

CLINICAL PATHOLOGY

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THIRD EDITION

With 12 Plates (10 Coloured) and 50 Illustrations
in the Text



LONDON

J. & A. CHURCHILL LTD

40 GLOUCESTER PLACE

PORTMAN SQUARE

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Printed in Great Britain

PREFACE TO THE THIRD EDITION

THE inclusion of much new matter without material addition to the length of the book has been mainly arrived at by re writing and re arranging much of the text and in particular the sections on the blood and parasitology. In achieving this we owe much to the generous assistance of our colleagues at the London Hospital.

Dr W W Woods is entirely responsible for the section on histology which he has again largely re written. Dr S P Bedson has contributed the articles on the filterable viruses the rickettsiæ and bacterial filtration. Dr I N Orpwood Price has written on the technique of the Kahn Test and Dr F C O Valentine has helped us in much of the revision and particularly in the section on the blood. We have also to thank Miss E M Burland for the preparation of the new index.

P N PANTON

J R MARRACK

PREFACE TO THE FIRST EDITION

THIS book represents an attempt to describe in a reasonable compass such laboratory investigations, whether chemical, histological or bacteriological, as have a practical bearing upon the diagnosis and treatment of disease, to give some account of their meaning and to assess so far as possible their value in practice

Many of the smaller text books of "Clinical Pathology" deal with the subject only in part, the complexity of methods in the larger works renders them more suitable to special investigators and it was felt that a book of intermediate size might be of use to the student and practitioner. The reduction in size has been mainly arrived at by avoidance of a reduplication of methods in preference to the omission of any essential branch of the subject. Where several methods for the same object are in use one or at most two, are described in full the remainder are omitted altogether.

Much of scientific interest has been necessarily sacrificed to the practical application of diagnostic methods, and the book has consequently no pretensions to consideration as anything more than an adjunct to clinical medicine.

No list of references is given, and the names of authorities, unless definitely associated with a particular reaction, are for the most part omitted.

In describing some of the more special investigations I have made use of among other works, Sequeira's "Diseases of the Skin," Plummer's "Practical Physiological Chemistry," Von Jaksch and Garrod's "Clinical Diagnosis," Sahli's "Diagnostic Methods" and Daniels and Alcock's "Tropical Medicine and Hygiene." The last work has also been of assistance to the artist in some of his drawings of the higher parasites.

I am originally indebted to Mr L. S. Dudgeon for many of

the methods of procedure recorded here and for numerous details of technique which have been of the greatest value to me in practice

The illustrations with very few exceptions have been specially drawn for this book from actual preparations Mr Shattock has kindly provided me with some of the specimens of intestinal parasites and Dr Turnbull with others

My colleague Dr Tidy has most kindly read and revised the manuscript and has assisted me with his advice upon numerous particulars Mr A C Hudson has also helped me freely with the final revision

1913

P N P

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CLINICAL PATHOLOGY

SECTION I

CHAPTER I

THE NORMAL BLOOD—THE BLOOD DISEASES

THE NORMAL BLOOD

THE study of the histology of the blood in health is essential to the appreciation of the changes which take place in disease. The following is a brief account of the normal blood.

The unstained blood (Fig 1 p 34) If a drop of blood immediately after being shed is examined under the microscope the red cells will be found to have arranged themselves in the form of long curved rouleaux, few if any red corpuscles remaining isolated. Occasional leucocytes will be easily recognised lying between the rouleaux, often in groups of 2 or 3. Small granular looking bundles of blood platelets will be made out usually in the near neighbourhood of a collection of leucocytes. After a few minutes a delicate network of fibrin appearing like a fine cob web will spread through the film, being densest in the vicinity of the platelets. Later the rouleaux will break up and the individual red cells will lose their shape and become crenated.

The red corpuscles are round or almost round biconcave discs having an average diameter of 7.5μ . They are oxyphilic and in stained preparations the centre of the disc is owing to the shape of the cell frequently paler than the periphery. The number of red cells is subject to considerable individual variation and is usually given as 5 million per c mm. For the normal adult Englishman the red cell count should be above this figure and commonly approaches 6 million per c mm, the number being somewhat less in the case of a woman.

The erythrocytes are derived from certain nucleated red cells of the bone marrow. The earliest recognisable precursor is the primitive megaloblast, a cell with a round or oval nucleus of which the edge stains deeply and the centre is filled with a

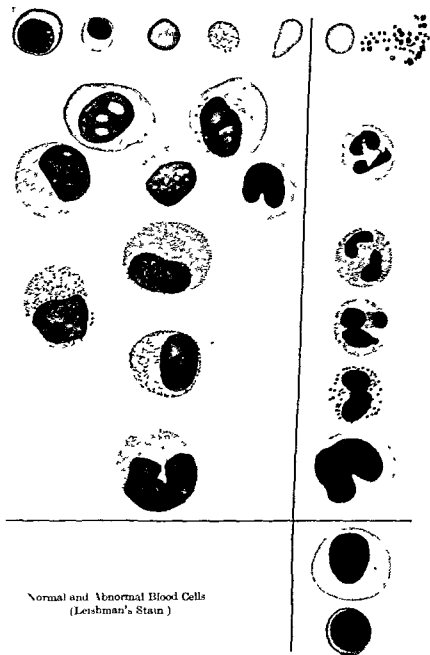
2 THE NORMAL BLOOD—THE BLOOD DISEASES

coarse mesh work of fine strands of chromatin, the cell cytoplasm being strongly basophilic. After a series of subdivisions the nucleus becomes stippled or "cart-wheel" in type and the cytoplasm—as it acquires hæmoglobin—polychromatic. From this cell is derived the normoblast with deeply staining pyknotic nucleus and oxyphil cytoplasm. Vital staining of the normoblast shows the nucleus to be lying in a reticular bed, and at a later stage the nucleus leaves the cell which is now puckered and irregular and still contains a thick ring shaped network of reticulum. The basic reticular substance finally breaks up and disappears, the cell regains its contour and becomes strongly oxyphil. The stages in the formation of the red cell are thus the primitive erythroblast or megaloblast, the later megaloblast with "cart wheel" nucleus, the normoblast, the reticulocyte, the erythrocyte. The earliest phase present in the normal circulation is the reticulocyte which forms about 10 per cent of the red cells.

The hæmoglobin is reckoned in percentages, and the standard of the amount of hæmoglobin present in a normal person is taken as 100 per cent. The percentage of hæmoglobin varies very little in the individual and any fluctuations greater than 10 per cent are significant. The normal standard of 100 does, however vary in different types of hæmoglobinometer, and the instrument used should always be recorded. It has become convenient by custom and for simplicity in calculating the colour index to record the hæmoglobin thus in percentages of the normal but it would be more in accordance with other estimations to return the actual percentage of hæmoglobin in the blood, and this ranges between 14 and 16 grammes per 100 c.c. of blood in the adult male. Minor variations within the normal range, or above it, are not detected easily with the hæmoglobinometers in common use, because readings of these instruments while simple in the lower parts of the scale are less sharp in the upper part, where a short distance on the scale represents a relatively large amount of hæmoglobin.

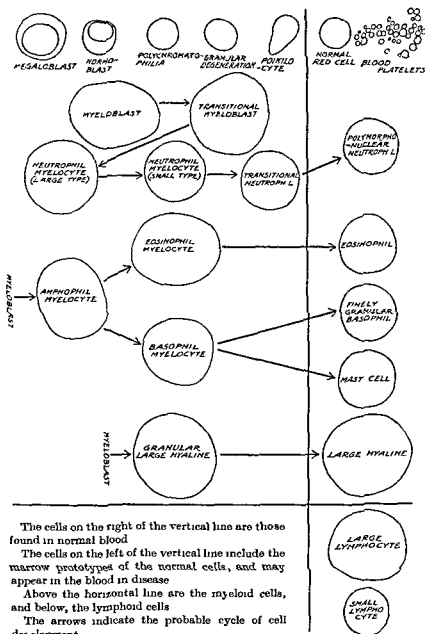
The colour index. By this is meant the index of the hæmoglobin carrying properties of the red cells. The index is obtained by dividing the percentage of hæmoglobin by the percentage of red cells per c.mm., and for this purpose the number 5 million per c.mm. is commonly calculated as 100 per cent of the normal for both sexes. For example, a specimen of blood containing 4 million red cells per c.mm., or 80 per cent of

PLATE I



Normal and Abnormal Blood Cells
(Leishman's Stain)

KEY TO PLATE I



The cells on the right of the vertical line are those found in normal blood

The cells on the left of the vertical line include the marrow prototypes of the normal cells, and may appear in the blood in disease

Above the horizontal line are the myeloid cells, and below, the lymphoid cells

The arrows indicate the probable cycle of cell development

the conventional normal number and 40 per cent of hæmoglobin, will have a colour index of $\frac{40}{80}$ or 0.5 indicating that each red cell contains half the normal amount of hæmoglobin. Since the normal red cell count is above 5 million per c mm the normal colour index is less than 1.0.

The size of the red cell can be calculated from the colour index when the cells are saturated with hæmoglobin as in normal blood or in pernicious anæmia. When the cells are not saturated the size can be measured directly, as in the Price Jones curve (p. 46), or from the volume index (p. 48) obtained from the hæmatocrit reading and the red cell count. Since the red cell is not a flat disc, alterations in the envelope or in the tension within it might lead to change in the curvature of the cell and in such an event the findings of the Price-Jones curve would not correspond with those of the volume index.

The white corpuscles. The normal number of leucocytes per c mm varies between 5,000 and 7,000, the average number being about 6,000. The number of leucocytes is subject to periodic fluctuations and varies slightly in the same individual at short intervals of time. It is doubtful if there is any appreciable leucocytosis following meals.

A differential leucocyte count gives the relative percentage of the different varieties and from the total leucocyte count the absolute number of each can be estimated.

The different kinds of leucocytes are recognised by the shape of the nucleus, the presence or absence of granules, the character of the granules when present, and the staining reaction of the cytoplasm.

The following varieties are met with (Plate I) —

(1) The finely granular oxyphil, or polymorphonuclear neutrophil—a cell with a nucleus usually trilobed and a feebly basophil cytoplasm thickly dusted with fine faintly oxyphilic granules. The normal percentage of this cell is from 50 to 65 of the total leucocytes.

(2) The coarsely granular oxyphil, or eosinophil—a cell with a nucleus similar to the above but usually bilobed and a definitely basophil cytoplasm filled with large, strongly oxyphil granules. The relative number is from 1 to 3 per cent.

(3) The coarsely granular basophil or mast cell—a cell with a nucleus usually bilobed, a cytoplasm which does not stain

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with the ordinary dyes, and coarse, scattered, strongly basophil granules. Relative number, 0 to 1 per cent.

(4) The finely granular basophil—a cell similar to the above but with fine basophil granules and with, as a rule, a frayed, degenerated nucleus. Rarely seen in normal blood.

(5) The large hyaline cell, or large mononuclear—a large cell with a characteristic notched or convoluted nucleus, and a reticulated cytoplasm which stains a greyish blue with Leishman's dye. This cell is an important phagocyte, is amoeboid, and is normally non granular. Relative number, about 5 per cent.

(6) The large lymphocyte—a cell with a round nucleus and a clear, pale blue cytoplasm, which frequently contains a few purple granules. Relative numbers, 5 to 10 per cent.

(7) The small lymphocyte—a cell with a round, deeply-staining nucleus which occupies almost the entire cell, leaving a narrow rim of deeply basic cytoplasm. Intermediate forms between the small and the large lymphocyte occur in normal blood. Relative numbers, 20 to 25 per cent.

The origin of the leucocytes (see Plate I). It is probable that the first five varieties of leucocytes are produced in the red marrow and the lymphocytes in the lymphoid tissues, including the deposits of such tissue normally present in the marrow. The primitive cell from which the four polymorphonuclear leucocytes are derived is a large cell with a round nucleus of open network and often containing two or three pear shaped nucleoli. The cytoplasm is non granular and intensely basophilic staining a greenish blue with Leishman's stain. This primitive mononuclear, non granular cell is called a myeloblast and in the course of its development granules appear in the cytoplasm clustered first around the nucleus and subsequently spreading to fill the entire cell. This granular cell is called a myelocyte and in its larger and more primitive form it has an eccentric nucleus (Cornu's myelocyte). The more mature form is smaller with a central nucleus (Ehrlich's myelocyte). Subsequently the nucleus becomes notched and incompletely divided to form the transitional polymorphonuclear cell, and, finally by further division the mature cell of the circulating blood. The type of mature cell evolved from the parent myeloblast depends upon the type of granule developed, if the granules are neutrophil, a neutrophil myelocyte and then a polymorphonuclear neutrophil cell is produced, if eosinophil

or basophil the mature cells formed are eosinophils or mast cells. Some myelocytes contain both eosinophil and basophil granules and may be called amphophilic myelocytes. The large hyaline or large mononuclear cell closely resembles a primitive cell found in the blood in some cases of acute leukaemia and usually called a monocyte. The nucleus of this cell may be more lobulated than that of the mononuclear and the cytoplasm may contain many fine neutrophil granules. These cells are produced in the marrow and possibly derived either from the primitive myeloblasts or from some other marrow connective tissue cell.

The primitive lymphocyte has a nucleus with an open network and a relatively large area of cytoplasm which is strongly basophilic. The most primitive forms cannot certainly be distinguished from the myeloblast.

Films made from the normal red marrow or from the blood of a case of chronic myeloid leukaemia show all gradations of cells intermediate between the myeloblast and the mature polymorphonuclear cell and the production of the successive stages in development can be readily followed.

Some acquaintance with the probable mode of formation of the normal leucocytes and red cells is necessary for an appreciation of the changes which may take place in the circulating blood in disease since the cells of the marrow may overflow into the peripheral blood stream. A moderate stimulus to the red cell formation as after hæmorrhage may lead to the appearance of additional reticulocytes and occasional normoblasts in the blood. The more fundamental disorder of pernicious anaemia may bring more primitive cells including the primitive megaloblasts into the blood in considerable numbers. A moderate overproduction of leucocytes as in acute inflammation may be accompanied by the appearance of transitional neutrophils and the small type of myelocytes. An extreme overproduction as in chronic myeloid leukaemia throws into the circulating blood every variety of marrow leucocyte including the mononuclear non granular myeloblasts. In acute myeloid leukaemia the normal process of development is arrested and only the most primitive cells are produced.

The platelets In addition to the various cells in a stained preparation of normal blood the platelets can be identified as basophilic rounded bodies of somewhat irregular shape occurring either singly or in small clumps throughout the film. They are probably produced in the bone marrow and

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possibly from the megakaryocytes, and they play a part in the formation of fibrin and the coagulation of blood. The number of blood platelets in normal blood varies between 250,000 and 400,000 per c mm.

THE BLOOD DISEASES

A number of morbid states are accompanied by more or less specific changes in the blood. They form a group of diverse conditions affecting the various cells of the blood and bone marrow and fall into three main categories corresponding to the three elements—the red cells, white cells and platelets—chiefly affected. Of those concerned mainly with the red cells, erythræmia is associated with a hyperplasia of the marrow and an over production of erythrocytes. Aplastic anæmia results from the destructive action of known or unknown poisons upon the marrow. Chlorosis and the microcytic anæmias are hæmoglobin deficiency diseases. Pernicious anæmia is due to a defect in the formation of the red cells. Hæmolytic icterus, sickle celled anæmia and hæmophilia are congenital abnormalities. Of those affecting the white cells the myeloid leukæmias are accompanied by abnormalities in the genesis of the marrow formed leucocytes and the lymphoid leukæmias by an overproduction of lymphocytes. An extreme deficiency of the platelets results in a form of purpura known as essential thrombopenia.

Erythræmia (splenic polycythæmia, Osler's disease, Vaquez's disease) is a rare affection. The condition is a chronic one attacking people usually of middle age, and the most noticeable clinical features in an advanced case are the striking plum coloured complexion, splenic enlargement, slow cerebration and the occurrence of venous thrombosis.

The blood obtained on pricking the lobe of the ear is almost black, and so sticky that it is difficult to obtain sufficient for a count without pressure, and it is impossible to make thin films with it. If the blood is taken into a tube, allowed to clot and centrifuged the red cells are found almost to fill the serum. The number of red cells is commonly about 9 million per c mm and has been known to exceed 12 million. The percentage of hæmoglobin may be 130 or over. The total number of leucocytes is frequently double the normal, and there is usually a relative increase in the polymorphonuclear neutrophils.

Occasional normoblasts can nearly always be found and the presence of these together with the polymorphonuclear neutrophil increase help to distinguish this disease from the secondary erythræmias resulting from the blood stasis of cardiac conditions. In primary erythræmia the blood volume is greatly increased.

Aplastic anæmia results from a destruction of the red marrow and to a lesser degree from internal hæmolysis. It is a rare condition and may result from exposure to radium the action of benzene or in the case of susceptible individuals from certain benzene derivatives such as tri nitro toluene. In many cases however no poisonous agent can be traced. The condition is rapidly fatal lasting as a rule from six weeks to six months. The blood changes are characteristic. The red cells rapidly diminish in numbers and the colour index remains high. Morphological changes in the red cells other than variations in size (anisocytosis) are absent. An extreme leucopenia develops the loss of white cells being confined to the marrow formed elements so that in the last stages the scanty white cells consist almost entirely of lymphocytes. The blood platelets are much diminished the bleeding time is prolonged and purpura may develop. The serum unlike that found in pernicious anæmia is colourless.

Chlorosis once a common now a rare affection is almost exclusively confined to young unmarried girls. The cause of the disease is unknown but it is possibly associated with some abnormal state of the female reproductive organs. The condition readily responds to iron but a return of the blood to normal is always slow and rarely complete unless a radical alteration is made in the surroundings or mode of life of the patient. The severe examples of chlorosis formerly seen often underwent spontaneous cure when the patient married and had children. The striking decrease in the incidence of chlorosis dates from the year 1914 and coincides with improvement in the conditions of female employment as well as in the general hygiene and mode of life of young women.

The main alteration detected by the routine examination of the blood is a loss of hæmoglobin. In a case of moderate severity there is little diminution in the number of red cells consequently the colour index is extremely low. In such a case the hæmoglobin would be about 40 per cent the red cells about 4 million per c mm and the colour index 0.5. In a

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severe case the hæmoglobin percentage falls very low indeed and the red cells may be considerably reduced though rarely to a number less than 3 million per cmm. In such a case occasional normoblasts and misshapen red cells or poikilocytes may be found in addition. The leucocytes are not altered in any characteristic manner but they may be slightly diminished and not infrequently the lymphocytes are relatively increased. In all cases the total blood volume is considerably increased and the oxygen capacity remains about normal the increase in volume being due to an increase in the amount of plasma.

The chlorotic blood picture presents no specific characters by which it can be certainly differentiated from other forms of hypochromic anemia which respond to iron it is not exclusively confined to the female sex and is identical with that of the microcytic anemias shortly to be described. Chlorosis is still considered to be a clinical entity but chiefly because of the association of the blood picture with the sex and age of the patient. The gastric acidity in chlorosis is normal.

Microcytic anæmia. There is a group of morbid states associated with hæmoglobin deficiency in which the red cells are microcytic and hypochromic appearing in the stained film as small cells with almost white centres and a deeper staining peripheral ring. The commonest form of microcytic anæmia might be described as the chlorosis of adult life. It is mainly confined to women and to the child bearing period relapsing with each pregnancy and disappearing after the menopause. It responds to iron in large doses. Unlike chlorosis it is commonly associated with achlorhydria or hypochlorhydria and occasionally with dysphagia. It has become more common as chlorosis has become more rare. The hæmoglobin deficiency may be severe but even minor losses may cause considerable disability and call for treatment. The diagnosis cannot be made without a blood examination which discloses the hæmoglobin deficiency, the relatively large number of microcytic hypochromic red cells and the low colour index. A similar blood state is found but much less commonly in man and occurs in certain nutritional disorders including celiac disease and some cases of sprue. The iron deficiency anemia of pregnancy, celiac disease or sprue resulting in a microcytic blood picture may be entirely obscured by a deficiency of the extrinsic or intrinsic factor and the opposite blood picture of megalocytic hyperchromic anemia may result. mixed states

may also occur and exceptionally a microcytic may alternate with a macrocytic anæmia in the same patient

Pernicious anæmia (Addisonian anæmia) is a morbid state associated with a characteristic, though not specific blood picture. It is rather more common in men than in women and may occur at any age, being most frequent in patients between thirty five and fifty years and extremely rare in early and young adult life. In untreated cases the average course is from two to three years, with spontaneous remissions in which the blood may return to normal and with almost invariably a fatal termination. In addition to the blood changes and the symptoms of anæmia, of which loss of vigour is the most prominent, the following associated conditions may be found. Periodic attacks of glossitis leading to an atrophic condition of the lingual mucous membrane complete achylia gastrica in over 95 per cent of cases and spinal cord lesions of the type and distribution known as subacute combined degeneration of the cord. The cord changes are often slight but occasionally form the predominant feature of the clinical condition. Similar nerve changes occur without anæmia but are also associated with achylia. A familial type of pernicious anæmia is not uncommon and corresponds to the frequently familial distribution of achylia. Achylia is often symptomless and only exceptionally proceeds to either anæmia or spinal cord disorder.

The blood changes in pernicious anæmia are as follows —

The red cells are greatly diminished often to a number in the near neighbourhood of 1 million per c mm.

The hæmoglobin is much diminished, but not in proportion to the decrease in the erythrocytes, so that the colour index is high and usually varies between 0.8 and 1.4. In a typical case the red cells would be 1 million per c mm, the hæmoglobin 25 per cent, and the colour index 1.25.

The leucocytes are characteristically decreased to between 3,000 and 4,000 per c mm.

In the stained blood the more important changes are found in the red cells many of which are abnormal in shape (poikilocytosis) and size (anisocytosis). Polychromatophilic cells are usually numerous, and other red cells are present which show basic granules in their cytoplasm—the so called granular degeneration. The average size of the red cells is above the normal as determined by the Price Jones curve (p. 46) or with the help of the hæmatocrit (p. 47). Very large red cells

are commonly numerous in the film and the megalocytosis is generally evident on comparison with a normal blood film. Nucleated red cells both normoblasts and megaloblasts may be numerous or may only be found after a careful search of these the former are found in any variety of severe anæmia but the megaloblasts and in particular those of the primitive type are almost diagnostic of this form of anæmia. Certain other but less noticeable changes are present in the leucocytes namely a relative increase in the small lymphocytes and to a less extent in the eosinophil cells. Occasional mast cells may be present together with 2 or 3 per cent of neutrophilic myelocytes usually of the small type. Old forms of polymorphonuclear neutrophils with six or more nuclear lobes can usually be found. The platelets are diminished.

Many of the changes described above may occur in any form of anæmia if sufficiently intense the changes most characteristic of pernicious anæmia being the high colour index with a low red cell count the presence of primitive megaloblasts and the megalocytosis.

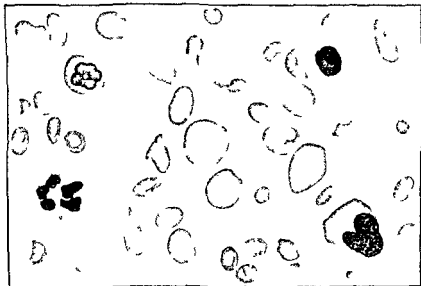
The conditions most liable to be mistaken for pernicious anæmia are those due to certain known organic causes which are capable of producing a severe anæmia and which may on occasion fail to give their usual physical signs and symptoms. Such are latent carcinoma and in particular carcinoma of the stomach. Addison's disease intestinal parasites and the anæmias following severe or small and repeated hæmorrhages.

The severe anæmias associated with infection by *bothriocephalus latus* and with sprue may give blood pictures indistinguishable from that of pernicious anæmia.

In the later stages of carcinoma metastatic nodules of growth may lead to considerable involvement of the bone marrow and to a striking blood state in which the red cell changes are even more extreme than in pernicious anæmia very numerous normoblasts and megaloblasts occurring. The colour index however is low. Such a condition has been given the name of *myelophthisic anæmia*. In the puerperium a severe state of anæmia is sometimes met with and the associated blood picture may so closely resemble that of pernicious anæmia that it is unwise to make this diagnosis in any patient with a recent history of pregnancy.

A further aid in the diagnosis of pernicious anæmia is afforded by the hæmatopoietic response to liver treatment. If

PLATE II



Leishman's Anemia
(Leishman's Stain)



Acute Inflammation
(Leishman's Stain)

half a pound of liver or its equivalent of extract is given daily by the mouth there is a quiescent period of seven to ten days followed by a rapid rise in the reticulocyte count which may reach to 25 per cent of the red cells, and the red cell count then rises and should increase by 500,000 per c mm per week until the normal figure is reached. The increase in red cells is accompanied by a corresponding rise in the hæmoglobin percentage. The blood can usually be maintained at a normal level by the continuous administration of the appropriate amount of liver for the individual for an indefinite period. The nerve lesions, if present, rarely progress under liver treatment, but it is exceptional for even the minor symptoms to disappear entirely.

The rationale of liver treatment is still obscure, but it seems clear that pernicious anæmia is due to the lack of some substance necessary for the proper maturation of the red cells. It is probable that this substance is manufactured by a previously unsuspected gastric secretion (the intrinsic factor) from an element of the food (the extrinsic factor) and stored in the liver and in other parts of the body for use in the bone marrow. In pernicious anæmia the intrinsic factor is missing, in other forms of megalocytic anæmia the extrinsic factor is wanting and may be replaced by marmite which is rich in vitamin B₂. The extrinsic factor is not, however, contained in yeast, and is therefore not vitamin B₂, but possibly a protein breakdown product.

Hæmolytic icterus in its usual form is a congenital familial disease affecting both sexes. The main clinical features are jaundice without bile in the urine, splenic enlargement, anæmia becoming periodically more intense, attacks of splenic pain and a tendency to the formation of gall stones. The routine blood examination shows an anæmia of varying intensity, which may be extreme. The colour index is commonly high, and nucleated red cells, chiefly normoblasts, may be very numerous. A feature of the stained film is the presence of many polychromatophilic cells which in vitally stained preparations are found to be reticulocytes. These cells may exceed 40 per cent of the total red cells and reach a figure higher than is found in any other morbid state. The diagnostic change in the blood is the presence of a high percentage of abnormally fragile red cells. This fragility is demonstrated by the ease with which the cells are hæmolyzed in hypotonic salt solutions. Normal

red cells retain their hæmoglobin in solutions of sodium chloride above 0.4 per cent the red cells in this disease are hæmolyzed in salt solutions up to 0.6 per cent or even stronger. The disabilities of this condition may be so trivial that patients may live to an advanced age without seeking medical advice. Far more commonly a serious degree of anæmia is found and the expectation of life is short, for these cases splenectomy is advisable, and results in diminution of the anæmia and disappearance of the jaundice. The red cell fragility remains after splenectomy. An acquired form of hæmolytic icterus is recognised, and has similar blood changes, but the degree of red cell fragility is rarely so extreme as in the congenital form.

Sickle-cell anæmia is a congenital and familial condition confined to the negro race. In the latent stage of the disease there is general glandular enlargement, frequently splenomegaly, and less commonly ulcers of the leg. In the active stage there is pyrexia, anæmia, hepatic enlargement and general glandular hyperplasia, and in this stage the blood changes are considerable. The red cells and hæmoglobin are much reduced and an appreciable leucocytosis is usual. The diagnostic feature is the appearance of the thin crescentic cells present in the stained film and in the fresh blood. In the latent stage blood changes are slight and no sickle cells are found in direct preparations, but if a drop of the fresh blood is ringed with paraffin and kept for some hours the crescentic red cells appear. No effective treatment is known and the patients rarely survive beyond early adult life.

Hæmophilia is a comparatively rare hereditary disease transmitted by unaffected females to males and characterised by extensive hæmorrhages, following trifling causes. Affected children rarely reach the age of maturity. The essential known morbid change is the alteration in the coagulation time of the blood. The normal coagulation time of the blood as estimated by Wright's method (p. 45), is very constant, and is almost invariably in the near neighbourhood of three and a half minutes. In hæmophilia the time is greatly prolonged, sometimes to as much as half an hour, and hæmophiles can be distinguished from other subjects of excessive hæmorrhage by this specific change in the blood. The length of the coagulation time fluctuates with the clinical condition of the individual, and during the remissions, when no bleeding occurs, the clotting of the blood may be prolonged by only one or two minutes. The

cause of this change is still unknown, being held by some to reside in the plasma, by others to depend upon the platelets, which are stated by Howell to be abnormally stable and to disintegrate more slowly than the platelets of normal blood

Chronic myeloid leukæmia (myelogenous leukæmia, spleno medullary leukæmia) Myeloid leukæmia occurs either as a chronic or as an acute disease and the two conditions have entirely different clinical states and blood pictures The chronic form may terminate, though rarely, as the acute condition which more often arises independently Chronic myeloid leukæmia is a rare affection occurring in males rather more frequently than females the average age of incidence being from twenty five to thirty five years It runs a comparatively chronic course terminating fatally in about two years The early symptoms are trivial and usually due to the great size of the spleen, which may reach down to the pelvis Myeloid leukæmia can readily be diagnosed by an examination of the blood The blood changes are as follows (Plate III) —

The blood flows freely on puncture and may continue to ooze for some hours The fresh blood under the microscope is quite characteristic, the leucocytes are so numerous that they appear to but practically never do, outnumber the red cells The majority of the cells are seen to be granular, the refractile granules showing quite well in the unstained blood The red cells as a rule show little change, fibrin formation is present, and the blood platelets are extremely numerous and massed into large clumps

The leucocytes are enormously increased, the usual number found in an untreated case being about 200,000 per c mm The red cells and hæmoglobin are both diminished, slightly in the early stages, greatly in the later periods of the disease The colour index is usually below 1.0

The stained blood film is prepared with some little difficulty owing to the large number of leucocytes, which necessitates the obtaining of a particularly thin film without pressure As the film dries it presents a curious greasy, semi opaque appearance due to the leucocytosis Over 90 per cent of the leucocytes are found to be granular and to consist of the following varieties —

Poly morphonuclear neutrophils .	about 40 to 50 per cent
Transitional neutrophils . . .	about 10 „

Neutrophil myelocytes (both small and large)	20 to 30 per cent
Eosinophils	5 to 10 ,
Eosinophil myelocytes	2 to 5 ,
Finely granular basophil cells	} 5 to 20 „
Mast cells	
Basophil myelocytes	2 to 5 ,
Amphophil myelocytes	1 to 3 „

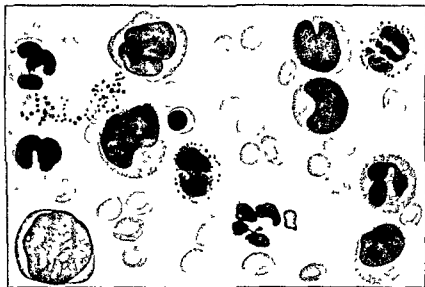
A proportion of the non granular cells consists of myeloblasts and many intermediate varieties of cells, which may be difficult to classify, are met with. Nucleated red cells both normoblasts and megaloblasts are usually found, and may be particularly numerous in cases with severe anæmia or after a hæmorrhage—a not infrequent occurrence in this disease.

The most important of the blood changes are—the great leucocytosis, the enormous preponderance of granular cells, the absolute increase of mast cells and eosinophil cells, the appearance of marrow prototypes in the blood, the presence of nucleated red cells.

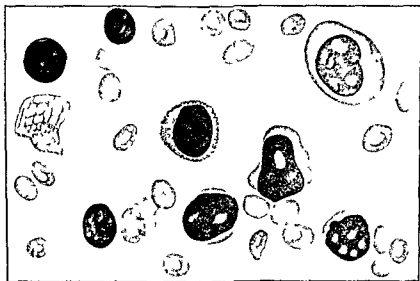
The blood picture of myeloid leukaemia may be completely altered by treatment, particularly by the administration of arsenic or the application of X rays. The most common results of treatment consist in a diminution in the size of the spleen accompanied by a decrease in the number of leucocytes, which may fall to the normal number or even below the normal. The differential count may be little altered, though exceptionally the stained film may show little evidence of disease, but as a rule occasional myelocytes persist and the mast cells remain relatively increased. Commonly the fall in the number of leucocytes is more marked than the shrinkage of the spleen and a patient may have a leucopenia with a spleen reaching to the umbilicus. Such a condition might be mistaken for splenic anæmia, and may persist for some months before a relapse occurs.

Chronic myeloid leukaemia usually progresses with temporary remissions to a state of anæmia incompatible with life. Less commonly, and on rare occasions following over treatment, the blood picture and the clinical state change to those of the acute disease, death following shortly after. The first indication of this change is the appearance of myeloblasts in rapidly increasing numbers at the expense of the more mature granular cells.

PLATE III.



Chronic Myeloid Leukemia
(Typical Film in course of 2 years duration) (Leishman's Stain)



Acute Myeloid Leukemia
(Myeloblastic Transformation from the same case as the above 1 year later
and 1 week before death)
(Leishman's Stain)

Acute myeloid leukæmia is a rare condition, which may occur at any age, but is commonest in young adult life. It is distinctly less rare among the Jews. Usually arising *de novo*, it exceptionally supervenes as the terminal state of the chronic disease. The clinical condition is associated with grave anæmia, fever, hæmorrhage from the mucous surfaces, purpura, enlargement of the liver, general lymphatic hyperplasia and splenomegaly. Both lymphatic and splenic enlargement may be inconspicuous and difficult to detect during life. The condition usually terminates fatally in about six weeks and is unaffected by treatment. It is improbable that recovery ever occurs.

The blood picture is diagnostic. There is a severe anæmia, often with a high colour index and associated with nucleated red cells in fair numbers. The white cells are greatly increased at first, and consist almost entirely (up to 99 per cent) of myeloblasts (Plate III). These are sometimes small cells with very little cytoplasm, and are difficult to distinguish from lymphocytes, in other cases the myeloblasts are large and typical. Less commonly the predominant cell may be the primitive monocyte. As the disease progresses the total number of leucocytes tends to fall and, at the time of death, there may be an actual leucopenia. Examples of this condition have been called pseudo leukæmia or aleukæmic leukæmia. The state is one of marrow exhaustion in which at first primitive cells alone are found, and subsequently production fails altogether.

Chronic lymphoid leukæmia (lymphatic leukæmia) is less commonly met with than myeloid leukæmia, is very rare in women, and may arise at any time of life, but most often in middle age and least rarely among the Jews. It is, in its typical form, a chronic condition which may last for four or more years. If it appears late in life, the course is usually more rapid and may terminate in a few months. The condition is accompanied by considerable enlargement of the lymph glands, usually confined at first to one or two areas such as the chain of cervical glands. The spleen commonly reaches to a hand's breadth below the ribs and may be considerably larger.

The blood changes (Plate IV) are characteristic. In addition to an anæmia of the secondary type there is a leucocytosis up to 200,000 per c mm or more, and the great majority of the cells are lymphocytes. The lymphocytes are usually of the

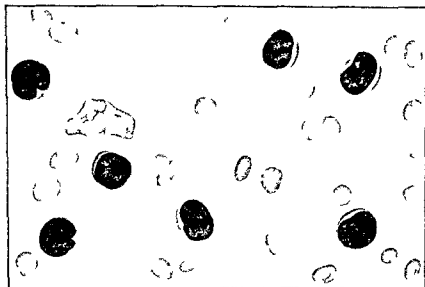
small variety, but sometimes of the large, and least commonly both types are represented. Whatever the type or types of cell present at the first examination, no alteration of note in their relative preponderance occurs in the course of the disease, but the total number may be diminished by X ray treatment. The disease usually terminates by a progressive anæmia or occasionally by intercurrent sepsis.

Acute lymphoid leukaemia accompanies a clinical state which appears identical with that of acute myeloid leukaemia and never appears to develop from the chronic lymphoid condition, the white cells in which always present points of difference from the myeloblasts. There are, however, no positive tests by which primitive lymphoblasts can be distinguished from myeloblasts, and until this is possible it seems preferable to describe the acute leukaemias with primitive non-granular cells as examples of acute myeloid leukaemia.

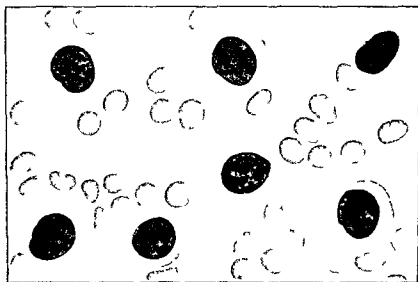
Essential thrombopenia is a condition which, owing to its characteristic blood changes, has been differentiated from other purpuric states. The clinical features are attacks of purpura with severe hæmorrhage, coming on often at intervals of several years, and splenomegaly. The blood changes, in addition to those of an ordinary secondary anæmia, are prolongation of the bleeding time without alteration in the coagulation time of the blood, failure of the blood clot to retract and extreme diminution in the blood platelets, which may fall to 10,000 per c mm or less. The failure of the clot to retract and squeeze out the serum can be readily demonstrated by withdrawing blood into a small bore test tube and letting it stand overnight. Splenectomy is followed by rapid amelioration of the clinical state and a return of the platelets to the normal numbers. Since the deficiency of the platelets is in all probability due to a marrow defect and the part played by the spleen is shared by the remainder of the reticulo-endothelial system there remains a danger that the platelets may again be destroyed, when the loss of splenic function has been compensated elsewhere and that a relapse may occur.

Diagnosis in all the morbid states described above is dependent upon an examination of the blood, and in the great majority of cases can be made with tolerable certainty provided that the fluctuating nature of the blood changes is recognised and that every advantage is taken of the clinical information derived from the patient.

PLATE IV



Chronic Lymphocytic Leukemia
(Lymphocytes - Small Type) (Leishman's Stain)



Chronic Lymphocytic Leukemia
(Lymphocytes - Large Type - from a case of 3 years' duration)
(Leishman's Stain)

CHAPTER II

THE SECONDARY BLOOD CHANGES—THE BLOOD CHANGES OCCURRING IN CHILDREN

THE secondary blood changes and their diagnostic significances are conveniently described under the following five headings, which denote the predominant change associated with various pathological conditions —

- (1) An increase in the number of white cells, or leucocytosis
- (2) An increase in the number of eosinophil cells, or eosinophilia
- (3) A decrease in the number of white cells, or leucopenia
- (4) An increase in the number of red cells, or polycythæmia
- (5) A decrease in the number of red cells, or oligocythæmia
- (1) Conditions associated with a leucocytosis

Acute inflammation is the most important underlying cause of an increase in the white cells in the secondary blood diseases. A typical example of the acute inflammatory process set up by one of the pyogenic organisms is met with in lobar pneumonia. The blood changes present in this condition are as follows —

The blood clots readily, and an increase in the blood platelets, together with an excessive fibrin formation, is seen in the fresh blood. A comparatively mild secondary anæmia is usually present. The leucocytes are markedly increased, often up to 20,000 or 30,000 per c mm. The number of the leucocytes does not fall with the temperature, but gradually diminishes as resolution occurs in the lung. The stained film (Plate II) yields the following differential count —

Poly morphonuclear neutrophils	. 80 to 95 per cent
Eosinophils	absent
Large hyalines	. 5 to 10 per cent
Small and large lymphocytes	. 2 to 10 „

The leucocytosis is seen to be due mainly to an absolute and relative increase in the polymorphonuclear neutrophils and to a lesser degree to an absolute increase in the phagocytic hyaline cells.

The complete, or almost complete, absence of the eosinophils is a very constant feature, and the reappearance of these cells is an indication that the inflammatory process is undergoing resolution.

Very occasionally in acute inflammation there is no increase in the leucocytes, and they may even be diminished, the relative proportion of the leucocytes, however, is altered in the manner described above. In a fatal case of extensive suppuration in the bile passages the total number of leucocytes was only 3,000 per c mm, but the polymorphonuclear neutrophils formed 90 per cent of the white cells. Such a blood picture occurs in a patient whose protective mechanism is failing to react to the infection, and is of grave prognosis.

Even more rarely both the total number of white cells and the relative number of granular cells may be greatly reduced, until the leucocytes fall to less than 1,000 per c mm and the polymorphonuclears may be almost absent. The condition is known as *agranulocytosis* and may be associated with a severe and often fatal septic and necrotic state of the buccal cavity. The failure to form granular cells is usually accompanied by severe red cell loss and the blood state is thus very similar to that of aplastic anæmia, a condition which may also be complicated by sepsis and necrosis.

The blood changes of acute inflammation are found in lobar pneumonia and accompany infections by any of the pyogenic organisms, such as the streptococci, staphylococci, colon bacilli and the like, and are of assistance in arriving at a clinical diagnosis. In a case of doubtful appendicitis an inflammatory blood count means that there is acute inflammation in the body, not necessarily in the appendix. It does not mean that actual suppuration has taken place and that immediate surgical interference is necessary on that account. The processes of acute inflammation and suppuration differ only in degree and so do the changes in the blood. When pus is pent up in the body the leucocytes tend to increase, when inflammation is resolving the white cells diminish and the eosinophils reappear. A rising leucocyte count in a case of appendicitis is evidence of abscess formation, a falling count with a diminution in the relative number of polymorphonuclear neutrophils is evidence of resolution. The clinical value of the blood examination therefore depends not only upon an enumeration of the total number of leucocytes present, but

also upon an estimation of the relative percentage of the cells found in the stained blood, and in addition a series of examinations may be necessary

Fevers Among the pathological processes giving similar changes in the blood are certain specific fevers, such as scarlet-fever, rheumatic fever, and the pustular stage of small pox

In **carcinoma**, particularly in carcinoma of the intestine, an inflammatory blood count is usual and is probably due to the infective processes set up by the growth (see also p 101)

Hæmorrhage, particularly when associated with intestinal lesions, is frequently followed by an inflammatory leucocytosis

In **tuberculosis**, in its usual and comparatively localised forms, the blood changes are very different, but in generalised tuberculosis especially with widespread glandular involvement, a typical inflammatory count is common If a patient with general glandular enlargement is found to have a high leucocytosis of the polymorphonuclear variety, the condition is extremely likely to be tuberculous

The presence of an inflammatory leucocytosis is always strongly suggestive of one of the above diseases, and helps to distinguish them as a group from any of the affections commonly associated with a leucopenia, such as influenza or typhoid fever A leucocytosis occurring in typhoid fever indicates that some complication is present—for example, perforation or an infection of the bile passages A continuous, high and rising leucocytic count suggests that an inflammatory process has gone on to pus formation A low total count with a high relative number of polymorphonuclear neutrophils is of grave prognosis

While an inflammatory leucocytosis is found with all acute septic lesions, an apparent exception must be referred to in tropical abscess of the liver. In this condition particularly when latent, leucocytosis is frequently absent, but it must be remembered that the "abscess" is in reality a chronic necrotic condition and does not contain pus If infection by pyogenic organisms occurs in the necrotic liver a leucocytosis supervenes

A leucocytosis resulting from an increase in the lymphocytes is met with in leukæmia, pertussis and glandular fever, but in practically no other conditions There is, however, a rare disease syndrome associated with a considerable leucocytosis, due to a relative and absolute increase of the small lymphocytes, and occurring in patients with a generalised erythrodermia

The striking red condition of the skin precedes the blood change and in the latest stages there is a lymphatic infiltration of the dermis. These cases appear to differ on the one hand from the generalised erythrodermia of mycosis fungoides which goes on to tumour formation but is unaccompanied by changes in the blood and on the other hand from those exceptional cases of leukaemia in which local infiltrations of the skin develop.

The Arneth index Morphological changes in the polymorphonuclears in the inflammations can be more closely studied by observation of their relative maturity. The more rapidly this cell is produced the more nearly it approximates to the mononuclear myelocyte and consequently fewer lobes are present in the nucleus. The *Arneth index* elaborates this principle. The cells are graded from 1 to 5 according to the number of nuclear lobes and the percentage of cells showing one or two lobes is added to one half the percentage of those with three lobes when this sum exceeds 50 the immature cells are in excess and the index is said to be shifted to the left.

(2) Conditions associated with an eosinophilia

Animal parasites are among the most important agents capable of producing an increase in the eosinophil cells. Any of the common intestinal worms may cause an eosinophilia and in particular the *ancylostoma* which produces in addition a severe anemia of the secondary type. *Trichinosis* hydatid disease filariasis and bilharzia disease also produce an eosinophilia. The increase in the eosinophil cells is usually accompanied by a leucocytosis and the relative percentage of the eosinophils may vary from 5 to 60 per cent or even more so that the total increase in these cells may be very considerable. In the case of hydatid disease an extreme eosinophilia accompanies any leakage of fluid from the cyst but the intact and particularly the fibrous or calcified cyst may produce no blood changes. An eosinophilia is not invariable in any of these parasitic infections and may be absent in uncomplicated cases. As an aid to diagnosis in doubtful cases the presence of a well marked eosinophilia is of value a negative blood examination is of less significance.

Skin lesions Eosinophilia is a condition associated with many varieties of affections of the skin. It is present in urticaria in psoriasis and in dermatitis herpetiformis. In the

latter condition the majority of the cells present in the bullæ may be eosinophils

In the specific fevers associated with skin eruptions and already mentioned as being accompanied by inflammatory changes in the blood a considerable eosinophilia is present in the early stages. In small pox the number of eosinophils may be very high in chicken pox measles and scarlet fever the condition is not so marked. Both in small pox and in chicken pox eosinophils are also present in the vesicles but disappear from the blood and from the skin lesions when suppuration takes place.

Blood diseases As already stated the eosinophils are increased in myeloid leukæmia and to a less extent in pernicious anæmia. In the latter disease an eosinophilia often of 10 per cent or more usually follows liver treatment. Following excision of the spleen in animals Ehrlich found the eosinophils increased after a considerable period a similar result has been noted to occur in human beings but as a rule the relative increase in these cells is very slight.

Spasmodic asthma and states of protein sensitisation such as serum sickness and the urticarias of food poisoning are accompanied by an eosinophilia in the blood. In uncomplicated asthma the sputum contains many eosinophils but the blood eosinophilia rarely exceeds 10 per cent.

Spring catarrh a rare affection of the eyes is associated with an eosinophilia in the blood and with large numbers of eosinophil cells in the conjunctival discharge.

(3) Conditions associated with a leucopenia

Chronic inflammation The blood changes associated with the chronic infective granulomata tuberculosis and syphilis are the reverse of those set up by the pyogenic organisms.

The total number of leucocytes is diminished usually to between 3 000 and 4 000 per c mm and the relative number of the lymphocytes is increased. The type of lymphocyte usually increased is the small lymphocyte a cell identical with or at any rate very similar to the lymphoid cell found in the giant cell system of tuberculosis and in the serous effusions associated with tuberculous and syphilitic diseases. In addition to the leucocytic changes there may be a considerable reduction in the number of red cells and in the percentage of hæmoglobin. The colour index is low.

A typical blood examination would give the following result —

Red cells	. . .	3,500,000 per c mm
Hæmoglobin	. . .	45 per cent
Colour index	. . .	0.6
White cells	. . .	3,000 per c mm

Differential count—

Polymorphonuclear neutrophils	. . .	40 per cent
Eosinophils	. . .	3 "
Large hyalines	. . .	4 "
Small lymphocytes	. . .	40 "
Large lymphocytes	. . .	13 "
		—
		100

In acute general tuberculosis, and particularly in wide spread tuberculous lymphadenitis, the blood changes are frequently those of acute inflammation, as has been mentioned under that heading.

Fevers Certain fevers are associated with similar blood changes, the more important being typhoid fever, influenza, malaria, and measles. In malaria and other tropical diseases the large mononuclears may be notably increased. In a febrile case of doubtful nature a complete white cell examination may be of considerable assistance in differentiating between the acute inflammatory and the non suppurative affections, as, for example, between a pneumococcal infection and influenza.

Hodgkin's disease, splenic anæmia and Banti's disease form a small group of morbid states of unknown etiology which do not give rise to any characteristic changes in the blood, other than the secondary anæmia associated with a leucopenia and a relative lymphocytosis common to so many conditions. In none of these affections can a diagnosis be made from a blood examination alone. In Hodgkin's disease the general glandular enlargement, together with the increase in the size of the spleen, may lead to a diagnosis of lymphoid leukaemia, and this can be negatived at once by a blood examination. The two conditions, however, have less clinical similarity than might be expected. In lympho sarcoma and in glandular tuberculosis there is no constant blood change capable of distinguishing the glandular enlargements from those of Hodgkin's disease. A

large number of nucleated red cells with the appearance of myeloid leucocytes would be in favour of sarcomatous or carcinomatous glands with other deposits in the bone marrow. The presence of a high polymorphonuclear leucocytosis would be in favour of tuberculous disease. A moderate eosinophilia is not uncommon in Hodgkin's disease, but it is too inconstant and insignificant to be of diagnostic value. The only certain method of diagnosis consists in the removal of a gland for histological examination, a proceeding which in the case of one of the discrete and superficial glands may readily be carried out under local anæsthesia.

Splenic anæmia on clinical grounds alone is difficult to distinguish from myeloid leukæmia but the latter disease can be recognised at once by a blood examination. In splenic anæmia the red cell and hæmoglobin loss may not be great, but the diminution in the number of leucocytes is more pronounced than in any other condition associated with spleno-megaly. The white cells may fall below 1,000 per c mm and are commonly between 2,000 and 3,000 per c mm.

Banti's disease is the name frequently given to a condition associated with gross disease of the splenic vessels and accompanied by splenic enlargement with cirrhosis of the liver, hæmatemesis, jaundice and ascites occurring in the later stages. Such a condition is hardly that of Banti's original description, and it must be recognised that cases of splenic anæmia and of Banti's disease constitute a group of varied states which have not yet been differentiated. The blood picture in all of them is similar.

In addition to the diseases already enumerated this type of leucocytic change is also met with in numerous conditions which affect more noticeably the red cells and hæmoglobin. A leucopenia with a relative lymphocytosis has already been stated to occur in chlorosis and pernicious anæmia among the primary blood diseases, they are also found in the anæmias of malignant growths, among workers in metallic poisons, and in numerous other morbid states.

A low colour index anæmia of greater or lesser degree associated with a leucopenia and a relative lymphocytosis is therefore a blood picture common to a great diversity of conditions. In young children a relative lymphocytosis is the rule, and even in the normal adult the proportion of lymphocytes to polymorphonuclear cells is greater than is commonly

stated, so that the diagnostic value of such a blood condition is mainly of a negative character indicating the absence of septic conditions rather than the presence of a particular clinical entity

(4) Conditions associated with an increase in the number of red cells (polycythæmia or erythræmia)

The number of red cells is capable of accommodation to circumstances, and is increased at high altitudes, during starvation, and temporarily after the removal of large quantities of fluid from the body, as after tapping an ascitic abdomen. Polycythæmia is accompanied by an increase in the hæmoglobin percentage and is found in the following morbid conditions —

Cardiac failure, accompanied by cyanosis and general venous stasis, leads to an increase in the number of red cells in the peripheral circulation. The number frequently varies between 7 and 8 million per c mm.

Congenital morbus cordis is almost invariably accompanied by a considerable polycythæmia, the number of red cells may exceed 8 million. Polycythæmia may be present when the cyanosis is by no means obvious, it is temporarily reduced by bleeding.

Erythræmia. The blood changes of this condition have already been described (p. 6).

(5) Conditions associated with a decrease in the number of red cells (oligocythæmia)

A decrease in the number of red cells is invariably associated with a decrease in the percentage of hæmoglobin, and it is this blood condition which is commonly referred to by the loose clinical expression "anæmia". If the unqualified term 'anæmia' is used at all it must be applied to designate a physical sign and never as the diagnosis of a disease.

The clinical recognition of the anæmic state is often impossible. Nothing can be more fallacious than the common idea that the colour of a patient's face, or even of his lips and conjunctivæ is any guide to the extent of his anæmia. Many sallow complexioned young women are given iron over periods of years to cure an anæmia from which they are not suffering, and one has frequently fallen into the error of expecting in a patient a considerable reduction in the red cells and hæmoglobin when there is little or none.

The erythrocytic mechanism of an adult in ordinary health

is very evenly regulated, and any serious drop in the red cells or in the hæmoglobin content is to be regarded as a definite indication of organic disease

The following are among the more important conditions associated with a diminution in the red cells and hæmoglobin —

Hæmorrhage is the simplest and most obvious cause of blood loss and is naturally followed at once by a proportionate loss in red cells and hæmoglobin. The red cells formed with great rapidity to replace those lost are deficient in hæmoglobin consequently the colour index quickly falls. After a single hæmorrhage the blood readily returns to normal in periods varying with the amount of blood lost and the recuperative powers of the individual. The blood lost by a normal person during an operation attended with considerable hæmorrhage should be replaced in from two to three weeks. Repeated small hæmorrhages may lead to a considerable degree of anæmia and if the bleeding has escaped observation the clinical state of the patient may come to resemble that of pernicious anæmia. The following may be given as an example of the condition in such a case

Fresh blood Rouleaux formation slight Fibrin formation normal or excessive Poikilocytosis present —

Red cells	2 500 000 per c mm
Hæmoglobin	30 per cent
Colour index	0.6

White cells normal in number or an increase of the acute inflammatory type

Stained blood Polychromatophilia present but not extreme Pale, microcytic cells numerous Occasional normoblasts seen but no megaloblasts White cells often show some increase in the relative numbers of polymorphonuclear neutrophils

Such a blood state is found not only after hæmorrhage, but in numerous conditions associated with 'anæmia' and is of the kind referred to as of the chlorotic or secondary anæmia type. The state differs from that of chlorosis in that the red cells are commonly more affected and the colour index higher, though these differences are too slight to permit of a differential diagnosis from the blood examination alone. It is of great importance to distinguish this secondary type of anæmia from primary or pernicious anæmia. The main differences in the secondary type are the rarity with which the red cells fall below

2 million a colour index less than 0.8 the small pale red cells and the absence of primitive megaloblasts

Metallic poisons Workers in arsenic antimony lead and similar metals may develop an *anæmia of the secondary type*. Basic granular degeneration of the red cells is especially characteristic of lead poisoning. Granular degeneration is present rarely in other diseases accompanied by a secondary *anæmia* but may be pronounced in pernicious *anæmia*. In acute lead poisoning such as is met with after attempted abortion granular degeneration of the red cells is usually extreme but in the chronic poisoning of workers in lead the red cell change is often not so easily demonstrated nor so constant as the appearance of the blue line on the gums it may often be detected by a methylene blue method (p. 47) when it is not visible in a Leishman preparation but by the former method fine granules may appear in some of the red cells of normal blood. Basic degeneration or more properly stippling of the red cells is probably not a degenerative process either of the cell or the nucleus. The fine basic granules appear to represent fragments of altered reticular substance.

The *cachexias* of carcinoma and tuberculosis are often associated with blood loss and consequently with a similar type of *anæmia* which is sometimes the first physical sign of serious disorder. In the absence of considerable or small repeated hæmorrhages these conditions together with syphilis nephritis diabetes myxædema Addison's disease and other chronic affections are commonly accompanied by *anæmia* of a different type. The form of *anæmia* is known as the simple microcytic the red cells being small but saturated with hæmoglobin and the colour index in consequence below normal but above that of the microcytic hypochromic *anæmias*.

Summary The red cell and hæmoglobin abnormalities in the blood diseases and in the secondary *anæmias* cannot at present be tabulated in any final or satisfactory manner but a partial classification may be attempted to include the commoner types of macrocytic and microcytic *anæmias*.

(1) **Macrocytic** (a) *Intrinsic factor missing*

Pernicious *anæmia* Sprue

(b) *Extrinsic factor missing*

(1) Lack of intake Malnutrition Tropical *anæmia* of pregnancy

(2) Lack of absorption Steatorrhœa Sprue (some cases)

(2) Microcytic. (a) Simple

Secondary to infections and disorders unaccompanied by hæmorrhage

(b) Hypochromic—due to iron deficiency

Chlorosis and the microcytic anæmia of women, chronic hæmorrhage Steatorrhœa and sprue (some cases)

Thus any one or more of the three chief factors necessary to red cell production may be missing, also the extrinsic factor and/or iron may be present in the food and not utilised owing to abnormal intestinal conditions. Recognition of the type of anæmia present will indicate whether liver, marmite (extrinsic factor) or iron is essential for treatment but it must be recognised that variations in the intake and the absorption of one or more of the necessary factors may not only give rise to different blood pictures in the same disorder as in sprue but may produce alterations of type in the same patient.

THE BLOOD CHANGES OCCURRING IN CHILDREN

Before considering the changes which may be found in the blood of infants it is necessary to appreciate that in children less than five years old the normal blood presents several differences from that of adults and tends to react excessively to abnormal stimuli.

In infants the total leucocyte count is high and the lymphocytes relatively numerous. Nucleated red cells are present at birth, and persist for several months after birth. The blood readily reverts to the foetal or bone marrow type in disease, and fluctuations in the number and character of the cells, such as would indicate the gravest disorders in an adult, may have little serious meaning in an infant. The blood forming mechanism in an infant has had much the same time to steady down as its heat regulating centre and both may be temporarily disordered by the cutting of a tooth.

The extreme characters of the fluctuations in the blood of infants renders the pathology of the blood obscure and any classification of the blood diseases of children almost impossible.

The blood changes of infants may be divided into —

(1) The blood diseases

(2) The secondary blood changes

(I) The blood diseases of infants

Pernicious anæmia and **lymphoid leukæmia** have both been described as occurring in infants, and it is possible that a very few of the reported cases are genuine examples of these conditions. The lymphocytosis of whooping cough may present a blood picture very similar to that of chronic lymphatic leukæmia, a condition which is almost non-existent in children. Occasional examples of fatal and severe anæmia of unsolved etiology met with in childhood are not unlike the Addisonian anæmia of adults. An aplastic type of anæmia is also rarely met with in the young. It is doubtful however, if either pernicious or aplastic anæmia of the types met with in adults arise during infancy, and such diagnoses should be made with the greatest reserve.

Myeloid leukæmia occurs in young children though very infrequently. In its chronic form only a few examples have been seen by us. In one case, a child of five years, reversion to the acute phase took place in a few months. Acute myeloid leukæmia is most frequently found among young adults and may affect quite young children. It usually takes a form to which the special name of **chloroma** has been given.

Chloroma may be regarded as a variety of acute myeloid leukæmia, with some peculiar clinical and pathological features. The blood picture is that of acute myeloid leukæmia, or as it is sometimes described (if the distinction between immature lymphocytes and small myeloblasts is not made) of acute lymphoid leukæmia. The special features are a tendency to occur in children or even quite young infants, the production of tumours about the skull, due to subperiosteal infiltrations by the myeloblasts, proptosis secondary to orbital infiltration and the presence of a green pigment in the periosteal swellings. These infiltrations are suggestive of malignant metastases and occurring in the viscera in other forms of leukæmia have led some to class these affections among the neoplasms.

In chloroma the periosteal swellings occur in situations very liable to induce errors of diagnosis, such as over the mastoid process or the frontal sinus leading to operative interference, or over the parotid region suggesting a diagnosis of mumps. The usual association of extreme pallor, bleeding from the gums and purpura should lead to a blood examination, when the condition can be at once recognised. The green pigment, which gives the condition its special name, is occasionally

met with in other forms of chronic and acute myeloid leukaemia

Splenic anæmia of infants (Von Jaksch's anæmia *Anæmia infantum pseudoleukæmica*) This is a disease affecting quite young children and accompanied by great enlargement of the spleen and moderate enlargement of the liver. The affected child may recover completely or may die or may improve and be left with a large spleen and may subsequently come into the category of Banti's disease. It is still a matter of dispute as to whether the disease constitutes a clinical entity or is a condition secondary to rickets or congenital syphilis. Rare cases are however met with in which no evidence of either disease can be found and in which the Wassermann reaction is negative. Such cases present a fairly typical clinical picture and a striking if somewhat varied blood condition so that it is reasonable to describe them for the present as examples of the primary anæmias of children.

The main features of the blood condition are as follows —

The red cells and hæmoglobin percentage are greatly reduced the former to a number between 1 and 2 million per c mm the latter to from 10 to 30 per cent.

The white cells are increased often to a marked degree and frequently number from 30 000 to 40 000 per c mm.

The stained film is very remarkable and may display every known kind of blood cell in considerable numbers. Nucleated red cells are always present and usually in excessive numbers. 200 may be seen while counting 500 leucocytes. Normoblasts are greatly in excess of megaloblasts. The relative percentage of the leucocytes is very variable and the predominant cells may be lymphocytic but are more frequently myeloid. Myelocytes especially the small type of neutrophilic myelocyte are numerous.

More rarely a great enlargement of the spleen may be associated with extreme red cell changes a high colour index and little or no change in the leucocytes.

Cases of splenic enlargement of this kind in children whether associated with congenital syphilis or rickets or of the Von Jaksch type have become much less common during the last ten years.

Erythroblastic anæmia Cooley has described a severe anæmia occurring in children of Mediterranean stock and accompanied by considerable splenic enlargement and certain

skeletal changes. The red cell disturbance is extreme and very similar to that found in von Jaksch's anaemia, nucleated red cells being particularly numerous.

Glandular fever is considered here, though it is by no means confined to children. It is met with in two forms, of which the milder affects children and young adults, appearing in communities in epidemic form. It is a mild febrile condition accompanied by glandular enlargement, is of comparatively short duration and has no mortality. The blood changes consist in a leucocytosis of from 10 to 40 000 per c mm, with a very high relative lymphocyte count, but the blood picture often fluctuates and the lymphocytosis may be missed at a single examination, nor may it appear in all patients in the same epidemic. The more severe form occurs more often in young adults and commonly starts with a sore throat, which may be followed by a morbilliform rash. The glandular swelling is generally most obvious in the neck and enlargement of the spleen is usual. The fever may be high and may persist for several weeks or even months. The general condition of the patient is as a rule remarkably good. The blood picture is almost diagnostic since, in addition to the lymphocytosis, a considerable proportion of immature large lymphocytes or lymphoblasts are nearly always present. In the majority of cases a positive or feebly positive Wassermann reaction is present, but for a short period only. It appears to be the latter or more severe condition to which the term *infective mononucleosis* has been given in the United States.

Haemophilia and *haemolytic icterus* may be considered among the blood diseases of infants. Both conditions are described in the previous chapter (pp. 12 and 11).

(2) The secondary blood changes

Rickets and congenital syphilis are among the most important primary conditions capable of producing extensive alteration in the blood of children. A considerable enlargement of the spleen and liver is frequently associated and a condition produced which may be very similar to that of splenic anaemia infantum. The degree of anaemia may be grave and nucleated red cells numerous. In some cases the leucocytes are greatly increased, in others there may be a leucopenia, with a relative lymphocytosis. The blood changes are very varied and rarely present to the degree seen in splenic anaemia from which the

secondary blood diseases are to be diagnosed partly by the minor enlargement of the spleen and the less obvious blood alteration but mainly by a recognition of the originating rachitic or syphilitic factor

Gaucher's disease A familial disorder accompanied by spleno megalý together with enlargement of the liver and some times of the lymph glands gives rise to no blood changes other than an anæmia of the secondary type Other spleno megalias of children associated with the names of Niemann Pick and Ebstein are similarly unaccompanied by any characteristic changes in the blood These conditions may not give rise to symptoms until early adult life

Pertussis A leucocytosis which may reach 60 000 per c mm with a high relative lymphocytosis is sufficiently constant in the course of whooping cough to be of considerable diagnostic value The lymphocytes are of the small variety and the increase takes place early in the disease

Infantile scurvy (Barlow's disease) is usually associated with a severe anæmia of the secondary type and as a rule with an inflammatory leucocytosis

Idiopathic steatorrhœa (the cœliac affection of Gee) may be accompanied by no blood changes but more commonly by a severe anæmia The red cell changes may be of the megalocytic and hyperchromic type similar to those found in sprue and responding to treatment by marmite or of the hypochromic microcytic type responding to iron In another proportion of cases the blood picture resembles that of the erythroblastic anæmia described by Cooley

Purpura in its various forms is not confined to children but occurs in them more commonly than in adults The most extensive purpura may not be accompanied by any characteristic changes in the blood As a rule there is a mild secondary anæmia and the leucocytes are unaltered The coagulation time is normal and there is no increased fragility of the red cells In essential thrombopenia (p 16) the bleeding time is prolonged and the blood platelets greatly diminished In children attacks may follow each other rapidly and lead to grave anæmia

CHAPTER III

THE METHODS OF EXAMINING THE BLOOD

THE ordinary routine examination of the blood includes the following investigations —

- (1) The unstained blood
- (2) The percentage of hæmoglobin
- (3) The estimation of the number of red cells to the cubic millimetre
- (4) The estimation of the number of leucocytes to the cubic millimetre
- (5) The stained blood

The essential apparatus comprises —

A microscope

A hæmoglobinometer

Blood pipettes and diluting fluid

A hæmocytometer

A special blood stain

Slides and cover glass

A dry swab ether and a surgical needle

The majority of these materials are described under their appropriate headings. The microscope is conveniently referred to here.

The microscope. A thoroughly reliable instrument is necessary not only for the examination of the blood but for numerous other pathological investigations in common use. For a student who is not hampered by motive of economy or by the previous possession of an inferior microscope the choice is not difficult if he bears in mind the following points. The microscope should have a firm base should be provided with a diaphragm and condenser a mechanical stage a triple nose piece for objectives having the focal distances of $\frac{2}{3}$, $\frac{1}{2}$ and $\frac{1}{4}$ inch and at most three eye pieces No. 1 ($\times 4$ diameters) No. 2 ($\times 6$ diameters) No. 4 ($\times 10$ diameters). The low power eye piece should be used for all ordinary work. The mechanical stage should be built into and form part of the framework of the microscope. It need not necessarily be graduated.

Thoroughly reliable microscopes are manufactured by a few of the leading English firms and there is no necessity to obtain an imported instrument. Discrimination must however be used in the selection of the $\frac{1}{4}$ inch objective since there is considerable variation in quality not only between lenses of different manufacturers but between those of the same firm.

If necessary the triple nose piece can be dispensed with and a double or even single nose piece used. A mechanical stage can be fitted to almost any microscope but is seldom entirely satisfactory. If much microscopic work is done a binocular instrument is greatly to be preferred.

To obtain the blood. Rub the lobe of the ear lightly with ether and allow it to dry. With as rapid a movement as possible make a deep puncture with a surgical needle. The novice is apt to press the needle deliberately into the ear giving the maximum amount of pain and obtaining the minimum quantity of blood. By a rapid stab sufficient blood can be obtained from a sleeping infant without awakening it. The best needle to employ is the ordinary straight Hagedorn No. 9. It should be sterilised before use by rapid passage through a flame. The special instruments of torture provided with nearly all forms of blood apparatus should be avoided. The drop of blood must be allowed to flow out and should not be squeezed out since pressure upsets the equilibrium between cells and plasma. Sufficient blood can rarely be got from the finger without pressure; it can always be obtained from the ear.

The unstained blood. Place a cover glass with one edge flush with the edge of a slide. Hold the two lightly in apposition with the thumb. Place the apposed edges against a drop of blood and the blood will flow between slide and cover glass. Examine within 10 minutes or so using the $\frac{1}{4}$ inch objective. All the available light should be thrown through the condenser and the diaphragm should then be closed down until the corpuscles stand out clearly. If the diaphragm is left widely open the cells cannot be seen at all.

Observe the extent of rouleaux and fibrin formation; the collections of blood platelets; the shape and size of the red cells and the relative proportion of white to red cells. Gross changes in the blood such as occur in the leukæmias can be recognised at once by this simple process; some of the minor changes can be observed in no other way and it should never be omitted. *Spirilla filaria* and trypanosomes can be readily

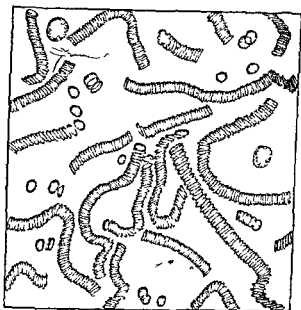


FIG 1—The Normal Unstained Blood

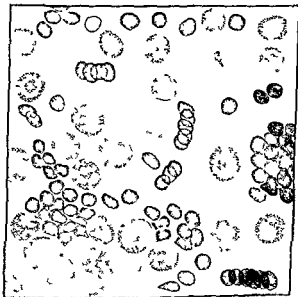


FIG 2—The Unstained Blood in Myeloid Leukæmia

recognised if present. Malarial parasites are more certainly identified with a $\frac{1}{2}$ -inch objective, the eye is attracted to them by the dancing of their pigment granules.

The hæmoglobin Some form of apparatus is necessary for the estimation of hæmoglobin, the blotting paper method of Tallqvist gives an approximate reading only. Numerous hæmoglobinometers are available but we are still without an entirely satisfactory instrument suitable for routine use. One of the following types is recommended.

The Dare hæmoglobinometer made by the Rieker Instrument Company of Philadelphia is a somewhat bulky and expensive instrument. The blood is taken from the lobe of the ear and is allowed to flow by capillary attraction between two slips, one of glass the other of porcelain held together by a screw. The film of blood is examined as soon as it is taken and before it has time to dry by comparing it with a circular wedge of tinted glass rotated by a screw. Illumination is provided by a candle or an electric torch. When the exact match between blood and colour standard is found, the reading of the standard in that position is given in percentages of hæmoglobin and in grams of hæmoglobin the normal standard of 100 being the equivalent of 16 grams of hæmoglobin per 100 c.c. In our hands the standard of normal males is well below the 100 per cent recorded by the scale provided.

The reading should be taken in a dark corner of the room, away from the light. Several rapid readings on the same blood specimen taken and compared should show little variation. Too long an observation tires the eye and is to be avoided. As with other forms of hæmoglobinometer readings in the lower part of the scale are easier to make than those in the upper.

Haldane's hæmoglobinometer is a modification of Gower's. A measured quantity of blood is taken into a graduated tube, saturated with carbon monoxide gas and diluted until it matches a standard solution in a second tube. Numerous modifications of this apparatus are in use, in Sahl's instrument the blood is mixed with $\frac{N}{10}$ HCl to form hæmatin hydrochloride and compared with a standard solution of the same substance. The Haldane apparatus has the disadvantage that carbon monoxide is required but is the more accurate instrument.

All the instruments referred to above are provided with leaflets giving adequate instructions. For ordinary clinical purposes one of the modern German modifications of Sahl's instrument can be recommended as compact, cheap, easy to use

and sufficiently accurate for most clinical purposes. A recent pattern called Helliges neo plane costs £2 10s. For special and more accurate investigations or for large laboratories the instrument recently devised by Campbell Smith and Holiday is probably the best available and is now manufactured by Messrs Tinsley & Co. The instrument is standardised against a solution of hæmoglobin of known strength and the concentration of hæmoglobin in a measured volume of blood is estimated by means of a photo electric device which measures the light absorbed in that portion of the spectrum occupied by Soret's band. The instrument is accurate to within 2 per

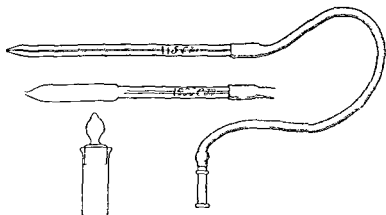


FIG. 3.—Strong's Pipettes and Mixing Bottle

cent and since no colour matching is required personal error has been reduced to a minimum.

The estimation of the number of red cells (1) Strong's method (modified) The necessary apparatus consists of two graduated pipettes a mixing bottle a diluting fluid and a hæmocytometer. The pipettes are graduated to hold 990 c mm and 5 c mm respectively. The 5 c mm tube has two marks close together. From the upper mark 5 c mm are delivered at the lower mark they are contained. The mixing bottle holds just over 1 000 c mm and is provided with a well fitting stopper.

The diluting fluid has the following composition —

- 85 gram sodium chloride
- 85 gram sodium citrate
- Commercial (40 per cent) formalin 1 c c
- Distilled water to 100 c c

The mixture must then be neutralised to methyl red. The formalin is an essential ingredient, since it fixes the drop for the white cell count on the slide and prevents it being washed off.

The method of use is as follows —

995 c mm of diluting fluid are measured into the mixing bottle, 5 c mm of blood are drawn up to the lower mark in the small pipette. The end of the tube is wiped, placed in the bottle, the blood blown out, and the pipette rinsed up and down with the mixture of blood and fluid. The mixing bottle is corked, shaken to prevent clotting and kept until it is convenient to count the cells. Before counting the bottle must be thoroughly shaken. After shaking, place the cover glass provided in position on the counting chamber and with a glass rod or a pipette allow a drop of the blood dilution to run along the centre table under the glass. If the counting chamber and cover glass are clean the fluid will run freely along the platform and there will be no air bubbles. The amount of fluid must be sufficient to fill the length of the platform and must not overflow into the side trenches. The slide is allowed to stand for a few minutes while the cells settle down, and is then placed on a

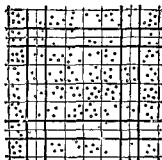


FIG. 4.—Field of Hæmocyto-
meter

microscope fitted with a No. 2 eye piece and a $\frac{1}{8}$ inch objective. The light is thrown through the condenser and the diaphragm is shut down in the same manner as when examining the fresh blood. The microscope must be vertical otherwise the red cells settle towards the lower part of the slide. The occasional leucocytes which may be present are readily distinguished from the red cells by their shape, colour, and the usual presence of refractile granules. The slide is moved until the ruled area of the central disc is found. This area is ruled in small squares, every fifth square in either direction having a line drawn through the middle of it. The area is in this manner marked off into 16 large squares, each of which contains 16 small squares bounded on every side with small double ruled squares. A large square occupies a field of the microscope. In counting the red cells only those in the single ruled squares are con-

sidered and as a minimum all the red cells in 4 large squares each consisting of 16 small squares are counted. In cases in which the red cells are much diminished all the 16 large squares should be counted. A certain number of the red cells will be found to impinge on the lines bounding the squares such cells should only be included when lying on the left hand and bottom lines of the squares. The average number of red cells per large square in health is about 110.

In order to calculate the number of red cells to the cubic millimetre it should be remembered that the dimensions of a small square are $\frac{1}{1000}$ of a cubic millimetre and that the blood has been diluted 200 times. The number of red cells to the cubic millimetre will therefore be $4\,000 \times 200 \times$ the average number of red cells per small square. If 400 red cells are counted in 4 large squares containing 64 small squares the number of red cells to the cubic millimetre of undiluted blood would be $4\,000 \times 200 \times \frac{400}{64}$ or 5 000 000.

(2) The Thoma Zeiss method. The apparatus consists of a combined pipette and mixing chamber a diluting fluid and a hæmocytometer. The diluting fluid employed may be the same as that described under Strong's method or a mixture may be used which contains a stain for differentiating the leucocytes. The staining mixture commonly employed is that of Tolson and has the following composition —

Methyl violet	0.25 gram
Neutral glycerine	30 c c
Distilled water	80 c c

Add to this a solution of —

Sodium chloride	1 gram
Sodium sulphate	8 grams
Distilled water	80 c c

Filter the mixture

To make the dilution draw up the blood in the pipette to the mark 0.5. Wipe the end of the pipette. Place the pipette in a bottle of the diluting fluid and draw up the fluid to the mark 101. Rotate the tube vigorously until blood and fluid are thoroughly mixed in the bulb of the pipette. The blood in the mixing bulb is now in a dilution of 1 in 200. Blow out the fluid in the capillary part of the pipette also a few drops of the diluted blood in the bulb and transfer a drop

to the platform of the counting side. Proceed as described under Strong's method. The disadvantages of this method are that it is difficult to be sure of a thorough mixing of the blood and fluid in the bulb, and that the contents of the pipette tend to leak out and necessitate the immediate counting of the cells. In Strong's method the pipette is more easily manipulated, the mixture is readily transported and can be counted at leisure, and the same mixture can be used without further apparatus for an enumeration of the leucocytes.

The estimation of the number of white cells. (1) Strong's method (modified). The apparatus required is the same as that for the red cells. The same mixture of 5 c mm of blood with 995 c mm of diluting fluid in a mixing bottle is employed. After thoroughly shaking the bottle draw up the mixture of blood and diluting fluid to the upper of the two marks on the 5 c mm pipette. Wipe the end of the pipette. Place a clean slide on the bench and hold the pipette vertically to the slide with the end of the pipette just resting on the centre of the slide. Blow out the fluid in the form of a drop, lifting the pipette and ceasing to blow just as the last portion of the fluid falls out. Allow the drop to dry. Stain with Ehrlich's acid hæmatoxylin (see p. 255) for 10 minutes, wash in tap water for 10 minutes, flood with spirit, and dry over the flame. Do not blot dry. When dry mount in cedar wood oil with a cover slip. (Instead of hæmatoxylin Leishman's stain, or any simple nuclear stain, such as carbol thionin may be used.) Place in the eye piece of the microscope a flat round metal disc with a central square aperture $\frac{3}{8}$ of an inch square, or, failing this, make a square hole in a piece of visiting card cut to the size of the eye piece. All that is required is to obtain a square field for counting the leucocytes in a round drop. Use the $\frac{1}{8}$ inch objective. Observe that the nuclei of the leucocytes are stained blue and the red cells are practically unstained. The edge of the drop is clearly defined. To count the leucocytes find the top segment of the drop and move the drop across the field from one side to the other. When the other side is reached mark a red cell on the bottom line of the square and move the drop down exactly 1 square field. Continue moving the drop backwards and forwards across the field until the entire drop has been covered and all the leucocytes have been counted. The number of white cells counted in a normal case would be about 150. The

dilution of the blood is 1 in 200 and 5 c mm of this dilution have been counted. The number of leucocytes in 1 c mm of the undiluted blood would therefore be 100 multiplied by $\frac{200}{5}$, or 6 000. In cases of leukaemia with an excessive number of white cells the enumeration of the leucocytes in a drop of blood diluted 200 times is too laborious and a further dilution of the blood is advisable. The further dilution may be made with a Wright's capillary tube (see p 174 Fig 20) in the following way. Make a mark on the tube and draw up to the mark 1 volume of the 1 in 200 dilution and 9 volumes of the diluting fluid. The blood is now diluted 1 in 2 000 times. Blow out the mixture into a watch glass mix thoroughly draw up 5 c mm of the mixture to the upper mark of the Strong's pipette and proceed as before. The number of leucocytes counted will have to be multiplied by 400 instead of by 40.

(2) *The Thoma Zeiss method.* The apparatus required consists of a special pipette a diluting fluid and a haemocytometer. The diluting fluid consists of a 0.5 per cent solution of acetic acid in distilled water with sufficient methyl green added to give the fluid a distinct green colour. The diluent dissolves the red cells and the methyl green stains the nuclei of the leucocytes. The blood is drawn up to the 0.5 mark on the pipette and the diluting fluid to the 11 mark. The pipette is manipulated as described under the enumeration of the red cells and the mixture is put up on the haemocytometer slide in the same manner. The dilution of the blood is 1 in 20. All the leucocytes in the entire set of 16 large squares are counted. To calculate the number of leucocytes per c mm of undiluted blood multiply the average number of leucocytes per small square (i.e. the total number of leucocytes counted in the 16 large squares divided by 256) by 20 times 4 000. The average number of leucocytes counted in the entire set of squares is only about 20 and the possible error is considerable. The advantage of this method is that it is more rapid than Strong's method.

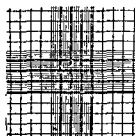
The Haemocytometer referred to above is that of Thoma as made by Zeiss or Hawksley and gives the ruling usually employed in the enumeration of red cells. Numerous other rulings are available and the most useful for general purpose is that of Neubauer (Fig 5). The central heavily ruled square is the equivalent of the Thoma ruling, and is used for counting.

the red cells either by the Strong or the Thoma method. The single ruled squares at the corners are each 0.1 mm deep and 1.0 mm square and can be used for counting the cells of a spinal fluid or the white cells of the blood and for these purposes the ruling is more suitable than the Thoma. For leucocyte counts the blood is taken into the Thoma diluting pipette as described above and the white cells in all the 4 large single ruled corner squares are counted. Each corner square has a capacity of $\frac{1}{10}$ c mm so that with a dilution of blood of 1 in 20 the calculation is—

$$\text{Number of cells counted} \times \frac{20 \times 10}{4} \text{ or } 50$$

A hæmocytometer is supplied with a double platform each with a Neubauer ruling. The red cell dilution can be put up on one platform and the white cells on the other so that both enumerations can be made consecutively on the same apparatus.

To clean pipettes. All blood pipettes should be cleaned immediately after use. It is sufficient first to suck water up and down the pipettes then alcohol and then ether. If particles of blood or dust have lodged in the pipette these should first be removed with a thread of fine silver wire. If the blood has been allowed to clot in the tube immerse the tube in 33 per cent acetic acid removing the blood with a fine wire at intervals. It may take a few days to remove a firm clot but the process may be considerably hastened by using glacial instead of 33 per cent acetic acid. If the fine end of a pipette is chipped or notched the pipette is broken and should be discarded.



F 5 Neubauer
Field

The stained blood. The materials required are clean slides or cover slips and a blood stain.

If cover-glasses are used for making the blood films it is essential that they should be clean and absolutely free from grease. The best quality of square cover glasses should be obtained and boiled in a wide evaporating dish or a sand bath for two hours in the following solution —

Sulphuric acid	60 c c
Potassium bichromate	60 grams
Distilled water	1 000 c c

Fresh solution should be added from time to time as evaporation occurs, and the glasses should be occasionally stirred with a glass rod. The glasses should then be transferred to distilled water and washed in it thoroughly with several changes. They should then be placed in absolute alcohol, and when required for use picked out with clean forceps and flamed.

Slides should also be of good quality, and can be cleaned in the same manner as the cover glasses. It is quite sufficient however, to rub them with very fine emery paper (the best emery paper for the purpose bears the trade symbol "Hubert 0000") and then to place them in absolute alcohol. When they are required for use wipe them dry with a clean cloth and then warm them in the Bunsen flame to drive off the last trace of moisture.

Numerous blood stains are available, but the student is advised to select one method and to confine himself to that for all ordinary work. Widely used stains are those of Jenner and Leishman in this country, and Wright's stain in America. All are excellent and of the three we prefer Leishman's stain.

Leishman's stain can be bought in tabloid form or in solution ready for use but it is advisable to buy the stain and the alcohol separately, and to make up in the following manner. To 0.2 grams of the stain in a well stoppered bottle add 100 c.c. of pure methyl alcohol. Shake well and stand for one week (preferably in the incubator at 37°C), shaking night and morning. The stain goes into complete solution, and, kept in a dark cupboard, it improves with time and will keep indefinitely in ordinary climates. It is essential that all bottles and measuring glasses used in the preparation of the solution should be chemically clean. The stain is best obtained from Grubler, but if this source is not available a good quality stain can be procured from G. Gurr, 136, New King's Road, Fulham S.W. 6.

To make the blood films either slides or cover glasses can be used. The latter give excellent results in skilled hands, slides give equally good results, and are to be recommended to those who are not in constant practice, since a bad film on a slide may be good in patches, a bad film on a cover-glass is useless.

To make a film on a slide place one end of the slide against the drop of blood taking care not to touch the skin of the ear. Place the slide flat on a smooth firm surface, such as a polished table and hold it in position with the thumb and first finger

of the left hand. With the right hand place the end of a second slide in the drop of blood and hold it there until the blood has run across the breadth of the slide. Draw the second slide slowly across the entire length of the first, maintaining an angle of about 45 degrees between the two slides. There should be no pressure whatever between the surfaces of the slides, and to ensure this the second slide should be held in the thumb and first finger of the right hand at about the level of their distal joints, the tips of the fingers being supported by the table. The more slowly the film is made, the thinner the resulting film. The even spreading of the film is assisted by previously warming the slide in the flame of a spirit lamp. As soon as the film is spread, the slide should

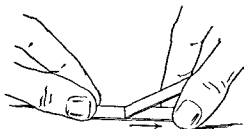


FIG. 6.—To make a Blood Film on a Slide

be waved vigorously in the air to ensure the immediate drying of the film, thus avoiding undue shrinkage of the cells.

No time must be wasted between the exuding of the blood drop and the completion of the film, since it is essential that no clotting should take place.

To make films on cover glasses, hold two glasses, one in each hand, by their corners. Place the centre of one glass against the drop of blood. Apply the centre of the second glass to the drop on the first. Hold the two glasses together until the blood has spread across the glass. Rapidly separate the two glasses. The thinness of the resulting film depends upon the size of the drop of blood and on the rapidity and evenness with which the glasses are separated. If the glasses stick at all, the separation has been unduly delayed and the films are useless.

To stain the film (1) With Leishman's stain

Cover the film well all over with the stain and leave for 30 seconds.

Add about twice the volume of *distilled* water to the stain

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Mix stain and water with a glass pipette until an iridescent scum forms over the surface and leave for 7 minutes

Pour off the stain and cover the film with distilled water only for 2 minutes

Wash off the water with fresh distilled water wipe clean the back of the slide and gently blot the film dry with clean blotting paper

To preserve the film mount when dry in Canada balsam with a thin cover glass

The film is fixed by the methyl alcohol in the first stage it is stained during the 7 minutes of the second stage and the colours are differentiated by the distilled water in the third stage

It is essential that the pipettes and beakers used should be first washed out with distilled water and that the water used should be *distilled*. The water must be neutral to methyl red

(2) With Jenner's stain

Cover the film with the stain and place a watch glass or inverted dish over the film to prevent evaporation

Stain for 4 minutes

Wash in distilled water until the film becomes a delicate pink tint

Blot dry

The *differential count* should be made with a $\frac{1}{2}$ inch objective and preferably with the help of a mechanical stage. Daylight should be used when available. Choose the thinnest and most even part of the film and count as a minimum number 200 leucocytes tabulating on a piece of paper each leucocyte under its proper heading

OTHER BLOOD EXAMINATIONS

The coagulation time A simple method of estimating the coagulation time of the blood is the lead pellet method. Cut a piece of capillary glass tubing into lengths of about 1 inch. The bore of the tubing should allow the free passage up and down it of a small lead pellet. After putting the pellet in the length of tubing round off both ends of the latter in a gas flame sufficiently to prevent the exit of the pellet without occluding the lumen. Put a dab of plasticine on the points of a pair of catch forceps. Half fill a beaker with water at 37°C and stand it on a white background. Apply one end of the tube to a

drop of blood which should flow into and completely fill the tube. Hold the tube with the forceps so that the plasticine retains the blood and place it in the beaker of water. Move the tube slowly up and down watching the passage of the pellet until it comes to a stop. The coagulation time is the interval between the filling of the tube and the arrest of the pellet and the normal range is from $1\frac{1}{2}$ to 2 minutes. By Wright's method a series of capillary tubes each holding 5 c mm of blood are filled and after stretching elastic bands over them to prevent the blood diffusing out are placed in a water bath at 37°C . The blood is clotted when it can no longer be expelled from the tube by blowing down it. The normal time by this method is $3\frac{1}{2}$ minutes.

The bleeding time may be gauged by pricking the lobe of the ear and blotting off the drop of blood every 30 seconds until no more blood exudes from the puncture. A fresh portion of the blotting paper is used for each successive blot and in this way a pictorial record of the bleeding time consisting of a series of blots is obtained. The average bleeding time in a normal individual is 2 to 6 minutes.

The fragility of the red cells. For the examination of the action of various strengths of salt solutions on the red cells no special apparatus is required. The necessary materials consist of 2 burettes, 10 large test tubes, sodium chloride, distilled water, a Wright's capillary tube provided with a rubber teat (p. 174 Fig. 20) and a number of small test tubes.

The procedure is as follows. Make up exactly a 1 per cent solution of sodium chloride in distilled water. Fill one burette with the salt solution, the other with distilled water. Run into the first large test tube 0.7 c c of water and 0.3 c c of salt solution (= 0.3 per cent salt) into the next tube 0.65 c c of water and 0.35 c c of salt (= 0.35 per cent of salt) and so on. A series of solutions from 0.3 per cent saline up to 0.75 per cent is thus obtained. Deliver an equal volume of each solution into a double series of the small tubes. Stand the two series of tubes in order in a rack. Make a mark about 2 inches from the end of the Wright's pipette. Obtain blood from the patient's thumb in the same way as for the estimation of the coagulation time. Blow out a volume of blood into each tube in the series. Repeat the process with the blood of a normal person in the second series of tubes. Shake each tube and stand the rack in the incubator at 37°C for 30 minutes. If no

incubator is available the tubes may be stood in warm water or even left at room temperature. In those tubes in which any hæmolysis occurs the supernatant fluid is tinged red and there is a corresponding deposit of red cells. Where hæmolysis is absent the *silt solution* remains colourless and the red cells form a deposit at the bottom of the tube. A sharper reading is obtained if the tubes are centrifuged or allowed to stand overnight at room temperature.

Size of red cells The mean diameter of red cells can be estimated from dried films, which for comparative purposes should always be fixed and stained by the same method. By means of a projection apparatus, adjusted for magnification of 1,000 diameters, the image of the microscope field is projected on to a sheet of paper lying on the bench. The red cells are outlined in pencil and the maximum and minimum diameter of each is measured with a millimetre scale. The mean of these two measurements is taken as the diameter of the cells and the mean diameter of 500 cells is considered to represent the mean diameter of the red cells for the specimen of blood.

Reticulocytes are stained in the following way —

With a Wright's pipette take up

1 vol. of 2 per cent citrated saline

1 vol. of 1 per cent cresyl blue

1 vol. of blood

Mix on a slide, draw up again into pipette and seal. After 15 to 20 minutes at room temperature, blow out a small drop on the centre of a slide mount with a cover glass and ring with vaseline. Count under an oil immersion lens, 500 consecutive red cells and enumerate separately as reticulocytes all red cells containing definite blue filaments. The number should not exceed $\frac{1}{2}$ to 1 per cent. Alternatively, the vitally stained blood can be drawn out on a slide as an ordinary blood film, dried in the air and then stained by Leishman's method.

Blood platelet estimation Place a drop of 2 per cent sodium citrate in normal saline on the skin and prick through the drop. Transfer a drop of mixed blood and solution to the centre of a slide mount with a cover glass and ring with vaseline. The mixture of blood and citrated salt should be made with the latter in considerable excess because if the red cells are too crowded the platelets cannot be counted. Under an oil immersion lens with artificial light and the diaphragm shut down make certain of the identification of the platelets.

Then count 1 000 red cells and the platelets lying among them. The normal ratio is about 1 platelet to 20 red cells. Count the total red cells by the normal method and from the platelet ratio estimate the total platelets per c mm. The normal number is from 200 000 to 400 000.

Stippling of red cells can be demonstrated by the following methylene blue stain. Methylene blue 1.5 gr. 1 per cent potassium alum sulphate in 50 per cent methyl alcohol 0.5 c cm. 1 per cent caustic soda in methyl alcohol 0.2 c cm. methyl alcohol 100 c cm.

Stain the film for 4 seconds. Wash rapidly in 0.025 per cent NaHCO_3 in distilled water. Wash in distilled water and blot dry.

The hæmatocrit. The form of hæmatocrit designed for use in these laboratories is supplied by Messrs Baird and Tatlock and consists essentially of a fine glass capillary tube graduated in hundredths. The blood is drawn into the capillary tube which is itself fitted into a brass outer tube having a screw cap with a tight rubber washer at each end and the whole is spun in a centrifuge for a fixed length of time. The relative volumes of plasma and cells can then be read off.

Technique. The blood is obtained by venepuncture and is immediately put into a small clean dry test tube containing a few particles of heparin and is shaken to prevent coagulation. Heparin has the advantage over oxalates that it does not cause shrinkage of the cells but has the disadvantage of being much more expensive. If oxalates are used to prevent coagulation an allowance must be made for the shrinkage in cell volume due to their action. By means of a rubber tube the blood is drawn up into the capillary tube so as to fill it exactly the ends being wiped gently if there is any excess. The end of the tube towards which the centrifugal force is going to be directed is first wiped with vaseline as an extra precaution to secure a non-leaking junction. The tube is inserted into the brass outer tube and the screw caps with their rubber washers are firmly screwed down. It is preferable to prepare two similar tubes of the blood and spin them against each other thus facilitating balancing and obtaining some measure of control in the readings. The tubes are balanced and placed in the centrifuge and spun for twenty minutes at two thousand revolutions a minute. At the end of this time the cells and plasma will have separated and their relative volumes can be read off. If the red cells are

also counted the *Volume Index*, giving the mean volume of the red cells, can be calculated by dividing the percentage of the normal hæmatocrit reading by the percentage of the normal red count

E g, Hæmatocrit reading = 30

Red cell count = 4 000,000

$$\text{Volume index} = \frac{30 \times 100}{45} \times \frac{5,000,000}{4,000,000 \times 100} = \frac{5}{6} = 0.83$$

That is, the red cells are $\frac{5}{6}$ of the normal size

The sedimentation rate is described in a subsequent chapter (pp 64 and 87)

CHAPTER IV

THE CHEMICAL COMPOSITION OF THE BLOOD

THERE is much more hope of detecting disturbances of metabolism by estimations of blood constituents than of those of urine, for the composition of the blood is normally maintained constant within fairly narrow limits, while that of the urine varies, of necessity, with the environment and diet of the patient.

Methods for measuring blood constituents have been developed very rapidly during the last twenty years, and since the first edition of this book appeared estimations of the greatest value for clinical purposes have come into general use.

Plasma proteins

	Fibrinogen	Globulin	Albumin
	Grams per 100 c.c. of plasma		
Normal	0.25 to 0.45	1.8 to 2.5	4.0 to 5.3
Pregnancy	0.50	"	"
Pathological			
Infections	0.5	3.0	4.0
Liver disease	0.15 to 0.50	—	—
Nephritis (with œdema)	0.60	2.0	1.8

The changes found in infections also follow the introduction of foreign proteins and, to a less degree, the absorption of exudates following aseptic operations.

Fibrinogen appears to be formed by the liver, high figures may result from stimulation of its formation, low figures from the reverse.

The reduction of albumin in nephritis is probably due to loss

of albumin in the urine, some degree of reduction is common in all types of nephritis, when the total protein falls below 50 per cent, or of albumin below 25 per cent, general œdema usually appears

Sugar

Normal . . .	0.09 to 0.12	gram per 100 c.c. of blood
After meals . . .	0.12 to 0.15	" " " "

Pathological

Mild diabetes . . .	0.15 to 0.30	" " " "
Severe diabetes . . .	up to 0.80	" " " "
Hypoglycæmia		
after insulin . . .	down to 0.03	" " " "

There is little doubt that the so called sugar in blood includes other substances that act as reducing agents, these, however, seem fairly constant, so that variations in the estimated concentration may be considered to measure variations of glucose. Some workers maintain that in the vessels glucose is mainly present in the plasma, but there is no doubt that after blood has been drawn and an anticoagulant added the glucose concentration is about the same in corpuscles and plasma.

The values found in diabetes vary greatly with diet and treatment, the figures given are merely an index of what may be found on a normal diet without insulin.

The blood sugar falls after the injection of insulin, reaching a minimum in about three hours. Symptoms of hypoglycæmia occur at different levels of blood sugar in different patients, but may be expected when the blood sugar falls below 0.06 per cent.

Large doses of insulin repeated at short intervals may have a cumulative action, and the blood sugar continues to fall for many hours after the last dose has been given.

A temporary increase of blood glucose may be produced by injection of adrenalin, by excitement or emotion, and by anæsthetics. These effects are probably the result of failure of storage of glucose or mobilisation of glucose already stored.

In normal persons glucose does not appear in the urine in appreciable amounts until the blood sugar rises above about 0.17 per cent, this level is called the renal threshold. It is not absolutely constant even in the same person and may be much lower than 0.17 per cent in persons who are in good health,

so that glucose may appear in the urine although the blood sugar is normal (renal glycosuria), the diagnosis of this condition is discussed in Chapter XX

Acetone bodies

Normal	0.000 to 0.006 gram	} Reckoned as β hydroxybutyric acid per 100 c c
Starvation	up to 0.04	
Diabetic ketosis	up to 0.15	

Retention of acetone bodies (acetone aceto acetic acid and β hydroxybutyric acid) in the blood usually occurs when the amount in the twenty four hours urine (p. 327) rises to about 10 grams but there is considerable variation depending on the efficiency of the kidneys. This retention is of greater clinical importance than the amount excreted.

The acids aceto acetic and β hydroxybutyric, when retained, cause a reduction of the plasma bicarbonate, if alkalis are not given but the bicarbonate reduction is not necessarily proportional to the amount of acetone bodies retained as other acids may be formed and retained in diabetic coma and reduction of plasma chlorides may to some extent, compensate for retention of acetone bodies.

The symptoms of diabetic coma are due, not to the reduction of plasma bicarbonate (acidosis), but to the actual acetone bodies particularly aceto acetic acid, the salts of this acid are as toxic as the acid itself.

Cholesterol

Normal	0.150 to 0.200 gram per 100 c c of blood			
Diabetes mellitus	up to 1.5	"	"	"
Nephrosis	up to 0.9	"	"	"
Anæmia	down to 0.08	"	"	"
Obstructive				
Jaundice	up to 0.7	"	"	"
Idiopathic	up to 0.4	"	"	"

The cholesterol concentration is approximately the same in plasma and red blood corpuscles. Elevations of the concentration, less striking than those tabulated, may be found in pregnancy, after feeding with fats, and in starvation.

In diabetes mellitus very high figures are only found in the more severe cases, and may be much reduced by treatment.

In nephritis the blood cholesterol is higher the more cases approach the type called nephrosis with œdema and little or no impairment of nitrogen excretion while as cases approach the chronic interstitial type with no œdema and impaired excretion of nitrogen normal figures are found

It has been maintained particularly in France that the high blood cholesterol is found in patients with gall stones but more trustworthy workers have failed to find figures appreciably above normal except in cases with obstructive jaundice

High blood cholesterol is sometimes found in patients with no apparent cause (idiopathic hypercholesterinæmia) Subcutaneous nodules containing cholesterol may occur in such patients and in diabetics with high blood cholesterol

An increase of the total fat and lecithin of blood above their normal figures (0.50 and 0.30 gram per 100 c.c. respectively) is usually found associated with high blood cholesterol this increase of fatty substances may give the plasma a milky appearance (lipæmia) and in extreme cases a cream like layer may form on the top of the plasma on standing

Urea

		Per 100 c.c. of blood.
Normal		0.020 to 0.030 gram
Pathological		
Retention		
Chronic nephritis	Moderate	0.03 to 0.08
	Terminal	up to 0.6
Heart failure		up to 0.07
Increased production		
Infections (pneumonia)		up to 0.07
Intestinal obstruction—acute		up to 0.20

Urea is the member of the nitrogen metabolites for which we have the most satisfactory methods of estimation and the greatest knowledge of its variations in disease Its concentration is slightly higher in the plasma than in the corpuscles

The high values found in infections and acute intestinal obstruction are probably due to toxic protein breakdown

As urea is slowly formed and rapidly excreted variations in the protein intake have little effect on the blood urea in normal persons but may produce great changes in nephritis The

significance of the blood urea in nephritis is further discussed in Chapter XVIII

Uric acid

		Per 100 c c of blood
Normal		0.002 to 0.005 gram
Pathological		
Retention	{ Nephritis	0.005 to 0.027 „
	{ Gout	0.004 to 0.016 „
Increased formation	Leukæmia	to 0.008 ,

Uric acid is estimated in the blood by colorimetric methods, it is uncertain how much of the colour developed is due to uric acid alone. The figures given are those found by Folm's (1922) method and are higher than those given by his original (1913) method. Variations of the diet quite apart from the purin intake cause considerable variations of the uric acid in the blood.

Uric acid estimations in the blood are valuable mainly in the diagnosis of gout, in this connection the high values found in other conditions particularly in nephritis must not be forgotten.

Creatinin

Normal	0.001 to 0.002 gram per 100 c c of blood
Nephritis	0.005 to 0.027 „ „

The objections to the methods of estimating uric acid in blood apply with still greater force to those for creatinin. Myers claims that 'blood creatinin' is of great value in prognosis in kidney diseases (see Chapter XVIII).

Creatin. The amounts found in blood and variations with disease are similar to those found for creatinin.

Non-protein nitrogen

Normal	0.025 to 0.030 gram per 100 c c of blood
Nephritis	up to 0.350 „ „

All the nitrogen containing substances which are not precipitated by protein precipitants, such as tungstic acid and trichloroacetic acid, are grouped together under the head non

protein nitrogen Urea is the main constituent of this group and variations of non protein nitrogen run closely parallel to those of urea

The non protein nitrogen unaccounted for by the constituents which can be estimated is called rest nitrogen naturally the errors in its estimation are large being the sum of the errors in the separate estimations it is therefore unsafe to draw conclusions from the amount of rest nitrogen found

Amino acids Blood contains about 0.007 per cent of amino acid nitrogen No significant changes have been found in any disease except necrosis of the liver

Ammonia The amount of ammonia in blood is very small—under 0.0001 per cent Methods of estimation are uncertain no unquestionable increase has been found in any disease and certainly not in nephritis with urea retention

Reaction of blood The hydrogen ion concentration (C_H) of a fluid is the number of grams of hydrogen ion present per litre In solutions of strong acids the hydrogen of the acid is almost entirely free as hydrogen ion while in solutions of weak acids most of the hydrogen is combined Water which may be thought of as a very weak acid breaks up slightly into hydrogen ion and hydroxyl ion so that at 37° C the C_H of

water is $\frac{1}{5\,500\,000}$ the reaction of body fluids lies near this

The figures for C_H involved in dealing with body reactions are therefore very cumbersome and the logarithmic notation of Sørensen is adopted almost universally the negative logarithm of C_H is called pH

thus if $C_H = \frac{1}{50\,000\,000}$

$$pH = -\log \frac{1}{50\,000\,000} = 7.30$$

In acid fluids the C_H is above that of pure water (and the pH below) and vice versa in alkaline fluids with increasing acidity the C_H rises and pH falls

It follows from the law of mass action that if the weak acid H_2CO_3 and its salts the bicarbonates are present in a solution

$$C_H = K \frac{\text{concentration of } H_2CO_3}{\text{concentration of bicarbonate}}$$

where K is a constant

The denominator and numerator of this fraction can vary independently and may therefore be considered separately

Bicarbonate

	Blood	Plasma
Normal	43 to 58	55 to 70 c c CO ₂ per 100 c c.
Slight acidosis	—	45 to 55 „ „ „
Moderate „	—	30 to 45 „ „ „
Severe	—	under 30 „ „ „
Moderate alkalosis	—	70 to 90 „ „ „
Severe „	—	90 to 120 „ „ „

As plasma is more convenient than whole blood for the estimation of bicarbonate, the plasma bicarbonate alone will be discussed. It is customary, owing to the original method used in its estimation, to express this as the number of cubic centimetres of CO₂ which are present in 100 c c of the plasma combined as bicarbonate.

The term *acidosis* will be used in this book to mean a diminution of plasma bicarbonate due to accumulation of acids (other than CO₂) or to diminution of base in the plasma, alkalosis to mean an increase of bicarbonate due to loss of acid from or increase of base in the plasma.

Acidosis may result from (a) excessive formation or ingestion of acid, (b) inefficient excretion.

(a) Occurs normally after severe exertion, when acids are formed in the muscles and pass into the blood. The chief pathological condition in which it occurs is ketosis, particularly that of diabetes, but it must not be imagined that acidosis invariably occurs in ketosis, still less that all forms of acidosis are due to ketosis. Before the introduction of insulin it was rare for patients with a plasma bicarbonate below 25 to recover.

A milder form of acidosis is seen after the administration of anæsthetics, particularly chloroform. Acidosis produced by the ingestion of ammonium chloride or calcium chloride may be used as a therapeutic measure.

(b) This form of acidosis, which occurs in patients with severely damaged kidneys, is the commonest found in hospital practice, more than half the patients whose blood urea is over 0.1 per cent have a plasma bicarbonate below 35. The acids retained in these cases are phosphoric, sulphuric, and other acids of undetermined nature.

An acidosis from loss of base has been described in infants with severe diarrhoea

When the C_H of the plasma is kept constant, the ratio $\frac{H_2CO_3}{BHCO_3}$ remains constant. When there is no interference with the gas exchange between the blood and the lungs, the H_2CO_3 in the blood is proportional to the alveolar CO_2 tension, so that the ratio $\frac{\text{alveolar } CO_2 \text{ tension}}{BHCO_3 \text{ in plasma}}$ is constant, in other words, changes in the plasma bicarbonate are accompanied by proportional changes in the alveolar CO_2 tension. But it must be remembered that this only holds if the two conditions, constancy of plasma C_H and free interchange in the lungs, are fulfilled.

When the plasma bicarbonate is reduced by acidosis, the urine is acid. If alkalis are given the urine begins to change towards the alkaline side when the plasma bicarbonate comes back to normal, this serves as the basis of an indirect way of measuring the plasma bicarbonate.

The opposite condition *alkalosis*, may result from ingestion of alkalis or from loss of acids. The first form may occur in the Sippy treatment of gastric ulcer, in the treatment of ketosis with alkalis, and in nephritis from moderate dosing with alkalis.

The second form occurs particularly in acute high intestinal obstruction and in other conditions in which profuse vomiting occurs. It is associated with a great reduction of the plasma chloride.

Alkalosis is much more dangerous than acidosis as the respiratory centre is depressed, lung ventilation and oxygen intake is reduced while, if the pH is raised, the hæmoglobin oxygen compound is rendered more stable so that the transfer of oxygen from blood to tissues is checked. When alkalis are used in the treatment of ketosis their administration should be stopped when the urine is no longer acid to methyl red.

However when the kidneys are damaged the reaction of the urine is not a safe guide.

Changes in the CO_2 tension of the blood cause the migration of chloride ions in and out of the red corpuscles, these migrations produce changes in the plasma bicarbonate, but such changes are small compared with those we have been discussing.

The carbonic acid concentration of the plasma is raised by

the excessive formation of CO_2 in violent exercise. It also varies with any interference with the respiratory exchange, heart and lung diseases may cause a slight increase, breathing air containing an excessive amount of CO_2 causes an increase, while hyperpnœa whether voluntary or involuntary, washes out CO_2 from the body and causes a decrease, unless excessive formation is occurring at the same time.

Hydrogen ion concentration

Normal	pH	7.3 to 7.5
Pathological	pH	down to 6.9 up to 7.8

The C_R of the plasma being proportional to the ratio of carbonic acid to bicarbonate, may change with changes of either. However changes of the bicarbonate are to some extent compensated by changes in the respiratory exchange, so that the C_R is kept fairly constant, for example, when the bicarbonate is reduced a certain amount of hyperpnœa usually occurs and the carbonic acid concentration of the plasma is reduced. When this compensatory process is sufficient to keep the C_R within normal limits, the process is called a 'compensated acidosis,' when insufficient we get an "uncompensated acidosis." Similarly, we may get a compensated or uncompensated alkalosis.

Compensation is often inadequate in the acidosis of nephritis, so that the pH may fall below 7.0, in the acidosis of diabetic ketosis compensation is much more successful a result of the great hyperpnœa so often seen in this condition.

Although interference with the CO_2 output may cause a rise of C_R in heart and lung diseases, it is more usual for hyperpnœa to occur of a degree sufficient to prevent accumulation of CO_2 in the blood or even to reduce it below normal and give a slight reduction of C_R .

A reduction of C_R , due to hyperpnœa, may be found in hysteria and particularly in hyperpiesia, the distressing hyperpnœa seen in hyperpiesia is not due to acidosis.

It must be remembered that reduction of blood C_R alone often gives rise to ketosis, which illustrates the absurdity of calling ketosis acidosis.

Chlorides Chloride concentrations are usually expressed in terms of sodium chloride, this is irrational, as the Cl in solution

is in the form of free ion, not bound up with any particular kation, but is so customary that it will be continued here

Since the concentration in the red blood corpuscles is only about half that in the plasma, great variations in the concentration in whole blood can be brought about by a change in the number of corpuscles without any other pathological change. For this reason it is advisable to work with plasma alone

	Reckoned as NaCl Grams per 100 c.c.	
	Red Blood Cor- puscles	Plasma
Normal	0.31	0.57 to 0.65
Acute intestinal Obstruction	—	0.40
Pneumonia	—	0.53
Nephritis	—	0.40 to 0.69

The plasma concentration varies little, the most striking changes the low figures found in intestinal obstruction and nephritis with great nitrogen retention are probably largely accounted for by vomiting. Retention of chlorides from excessive intake or diminished excretion is usually accompanied by retention of water sufficient to prevent any great rise of Cl in the body fluids.

In nephritis plasma chlorides above 0.63 per cent (as NaCl) are rare, particularly in cases with oedema. The low figures are found only in cases with great nitrogen retention.

Inorganic phosphates

Normal	0.003 gram per 100 c.c. of blood
Infants	0.0055 "
Rickets	0.002 " "
Nephritis	0.020 " "

The concentration of inorganic phosphate is approximately the same in red blood corpuscles and in plasma.

The figures normally vary with the time of year, being higher

in summer, lower in winter Low figures are usually found in active rickets

In nephritis the inorganic phosphate concentration runs roughly parallel with the blood urea, its value in relation to prognosis is discussed in Chapter XVIII

Blood also contains organic esters of phosphoric acid, which are almost wholly confined to the corpuscles, though of great physiological interest, they have not yet been sufficiently studied in disease to be of clinical importance

Total base and sodium The total base of the plasma lies between 0.150 and 0.160 gram equivalents per litre and is very constant, this base consists principally of sodium (about 0.33 gram per 100 c.c.)

Potassium also varies little from its normal figure (0.020 gram per 100 c.c.), in nephritis an increase up to 0.030 has been described

Calcium

Normal	.	0.010 to 0.012 gram per 100 c.c.		
			of plasma	
Nephritis with low proteins		0.007	"	"
Nephritis with phosphate retention		0.006	"	"
After parathyroidectomy		0.005	"	"
Failure of absorption		0.007	"	"
Rickets		0.007	"	"
Osteitis fibrosa	.	0.015	"	"

The red corpuscles contain much less calcium than the plasma—not more than 0.003 gram per 100 c.c.

Calcium in the plasma is partly present as ion, partly as an un-ionised protein compound. When the total proteins of plasma are reduced, as in some cases of nephritis, the un-ionised fraction alone may be reduced, this reduction appears of little significance. Reduction of plasma Ca in the other conditions affects the ionised fraction as well and appears to be the cause of the tetany seen in the three latter conditions.

Low figures in nephritis, unaccounted for by the reduction of protein, and therefore affecting the ionised fraction, occur only when the inorganic phosphate rises above 0.008 per cent, and are usually associated with muscular twitchings.

Increase of plasma calcium (both fractions) up to 0.018 per cent occurs after injection of active parathyroid extract. Such an increase may be accompanied by severe general disturbances, and it is not advisable to raise the level above 0.015 per cent.

Magnesium Although variations from the normal figure, 0.002 per cent, have been found in various conditions, these are not constant, and no clinical importance can as yet be attached to its estimation.

Plasma phosphatase

Normal	0.10 to 0.21 units
Rickets	0.62 to 1.7 "
Osteomalacia	0.31 to 1.8 "
Osteitis fibrosa	1.1 to 2.5 "
Osteitis deformans	0.65 to 3.2 "
Exophthalmic goitre	0.27 to 0.75 "

An enzyme is found in animal tissues, particularly the epiphyseal zone and periosteum of bone, intestinal mucosa and kidney, which will hydrolyse esters of phosphoric acid. A great increase is found in diseases affecting the bones. A sample of plasma is said to contain one unit of phosphatase when 1 c.c. of the plasma will liberate 1 mgm. of inorganic phosphate (expressed as P) from excess of sodium β -glycerophosphate solution in 48 hours at 38° C. and at pH 7.6.

Bilirubin

	Amount	Van den Bergh Reaction
	Per 100 c.c. plasma	
Normal	0.0 to 0.5 mgm	Indirect
Pernicious anæmia	0.7 to 7 "	Indirect or delayed
Simple obstructive jaundice	Up to 50 "	Direct
Pneumonia	Up to 4 "	Delayed or indirect
Cirrhosis of liver	0.4 to 6 "	Indirect or biphasic

Bilirubin is formed from the hæmoglobin of red blood corpuscles, both in the liver and in the reticulo-endothelial system elsewhere. The bilirubin so formed circulates in the blood and is excreted by the liver. Excess of bilirubin in the

blood may result from excessive formation owing to increased breakdown of red corpuscles, or from interference with excretion owing to damage to the liver or obstruction to the bile passages. There is some difference in the form in which bilirubin circulates in these two conditions, when the excess of bilirubin is due to obstruction the bilirubin in the plasma will react at once with diazo benzene sulphonic acid (direct Van den Bergh reaction) but when the excess results from excessive breakdown the reaction is delayed or does not take place unless the proteins of the plasma have been precipitated by alcohol* (delayed or indirect reaction). This difference in the state of the bilirubin appears to result from passage through the liver cells and, therefore, with liver damage a mixture of the two forms of reaction may be expected (biphasic reaction).

The presence or absence of excess of bilirubin in the plasma may be of value in differentiating hæmolytic from secondary anæmias, while the state of the bilirubin in the plasma may help in the diagnosis of the cause of obscure cases of jaundice. Excess of bilirubin can be seen in the plasma or serum before yellow pigmentation of the sclerotics is noticeable, still more before bile pigments can be detected in the urine.

Bilirubin in serum is often expressed in units. One unit is equivalent to 0.5 mgm. of bilirubin in 100 c.c. The use of these units merely leads to confusion.

CHEMICAL CHANGES IN HÆMOGLOBIN

Carboxyhæmoglobin, the compound formed by carbon monoxide and hæmoglobin, is much more stable than that formed by oxygen and hæmoglobin, it cannot be reduced by the tissues or the reagents commonly used, and a small concentration of carbon monoxide will combine with a large part of the hæmoglobin of the blood. An atmosphere containing 0.02 per cent. of carbon monoxide, if breathed for some hours, will convert about one fifth of the hæmoglobin of the blood to carboxyhæmoglobin and produce headache and nausea, 0.04 per cent. will convert nearly one half and produce collapse, while 0.1 per cent. will convert nearly two thirds and produce coma and death. The time taken to produce these final concentrations is shorter the more active the subject. Carboxy-

* For details see under methods.

hemoglobin is cherry red and its spectrum very similar to that of oxyhemoglobin

Carbon monoxide poisoning may occur after exposure to coal gas, the fumes of charcoal stoves, or the exhaust gas from motors in confined spaces, it may also occur in people who are

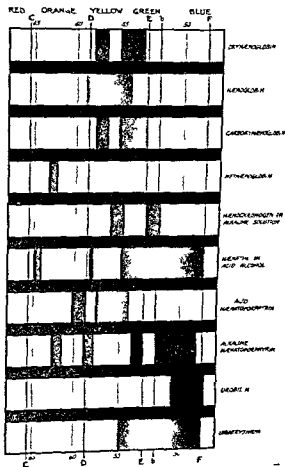


FIG 7—Absorption Spectra.

trapped in burning houses Should death occur, the carboxy hemoglobin is not reduced, as is oxyhemoglobin, so that the body has a pink tinge and the blood is bright red

Nitric-oxide-hemoglobin is formed by the action of nitrites on reduced hemoglobin It may be produced after death as a result of the formation of nitrites by bacteria, Haldane

suggests that it may be formed during life in infections with nitrifying bacteria when oxygenation of the blood is much reduced Nitric-oxide hæmoglobin closely resembles carboxy hæmoglobin in spectrum and other properties

Methæmoglobinæmia may be produced by certain drugs particularly phenacetin and antipyrin even when taken in medicinal quantities by susceptible patients The same condition may arise among workers in aniline dyes and nitro glycerin factories from the inhalation of nitrobenzol compounds and may follow poisoning by chlorate of potash and by pyrogallie acid In the more advanced cases the patients are markedly cyanosed and the colour of the blood brownish when only slight the condition is very difficult to detect The characteristic absorption spectrum of methæmoglobin is a narrow sharply defined band in the red (between C and D)

Sulphæmoglobin is said to occur in the blood in a condition called *Sulphæmoglobinæmia* or *Enterogenous cyanosis* The condition is at least extremely rare one much described case examined by one of us had methæmoglobinæmia and was taking acetanilide

The condition is supposed to be produced by sulphuretted hydrogen absorbed from the intestine which is improbable since sulphuretted hydrogen kills by its action on the nervous system in quantities insufficient to affect the blood The spectrum of so called sulphæmoglobin resembles that of a mixture of methæmoglobin and oxyhæmoglobin

Cyanhæmoglobin a red pigment is formed by the action of cyanides and hydrocyanic acid on hæmoglobin The amount required to form this compound in the body is greater than that required to kill it will therefore not be found during the life of a patient nor if death has been due to inhalation of hydrocyanic acid as in the latter case not more than the lethal dose can be absorbed When cyanides are swallowed they may diffuse from the stomach after death in sufficient amount to form the pigment especially in the dependent parts

In cyanide poisoning the uptake of oxygen by the tissues is arrested and oxyhæmoglobin is therefore not reduced This is the chief cause of the pink colour of the bodies of persons killed with cyanides

Cyanhæmoglobin has a spectrum resembling that of reduced hæmoglobin it is not affected by reducing agents in the cold

7 **RATE OF SEDIMENTATION OF RED CORPUSCLES**

An increase of the ratio of fibrinogen and serum globulin to serum albumen leads to an increased rate of sinking of the red blood corpuscles. The increase of the ratio may be due to an actual increase of fibrinogen, serum globulin, or both or to a reduction of serum albumen. The rate of sinking is also increased when the number of red corpuscles is reduced. Using the method described in the next chapter the corpuscles in normal blood sink slowly to about 83 in an hour. In pregnancy a great increase in the rate is noticeable, as the corpuscles sink to about 70 in the first 15 minutes and settle completely in about 45 minutes. In malignant disease and active tuberculosis the level sinks to about 60 in 15 minutes, and even more rapid sedimentation may occur in acute infections and nephrosis. Although of no diagnostic value an increased rate of sedimentation may be regarded as evidence that some disease is present.

CHAPTER V

THE CHEMICAL EXAMINATION OF THE BLOOD METHODS

It is as a general rule more satisfactory to obtain blood for chemical analysis by venepuncture rather than by finger prick methods. Since the blood obtained by venepuncture can be measured out at leisure and larger amounts can be used much greater accuracy can be obtained also several different constituents can be estimated in the same sample. Some consideration also is due to the patient one of the authors after considerable experience of both methods of bleeding can speak with some heat in favour of venepuncture. It is however advisable to practise finger prick methods as they are useful on occasions when no veins can be found as in very small children or in very fat persons also when repeated samples have to be taken from patients whose veins are not easy to find since venepuncture is often followed by some effusion which results in increased pain and difficulty at later attempts.

For venepuncture a 10 c.c. all glass syringe with a needle (bore 16) are suitable small needles may be used for very small veins. A short needle (bore 14) with a short piece of rubber tubing attached is convenient when large amounts of blood are required but it is difficult to avoid spilling a little blood. The method of obtaining blood by venepuncture is described on p. 224.

As soon as sufficient blood has been obtained it should be squirted into a tube containing potassium oxalate unless required for uric acid or calcium estimation. It is convenient to keep a supply of test tubes prepared by adding to each 0.25 c.c. of 5 per cent potassium oxalate solution stoppering with cotton wool and drying in an oven. Each of these tubes contains enough potassium oxalate to check the clotting of 10 c.c. of blood. After the blood has been squirted in the tube should be inverted to mix the oxalate with the blood. Citrates

should not be used, as they are very poor anticoagulants and interfere with several estimations

For the finger prick method the patient's finger or thumb is stabbed as described on p 174, it is advisable to sprinkle a little potassium oxalate on the skin before pricking When a fair sized drop of blood has appeared, it is allowed to run into a horizontally held 0.2 c.c. pipette, to just above the mark, the surface of the blood is then brought down to the mark by tapping on the thumb nail, and the excess of blood is wiped from the point of the pipette with a piece of filter paper, the blood in the pipette is blown out into distilled water, and the pipette is washed out by sucking the water up and blowing out twice It is essential that the pipette should be perfectly clean, it should be cleaned after use with sulphuric acid bichromate mixture *

Larger quantities of blood can be obtained from babies by stabbing the heel with a small tenotome and letting the blood drop into a small dish containing potassium oxalate Blood may also be obtained from the external jugular vein, for this purpose a very sharp needle is necessary, as the vein cannot be fixed

In all estimations made on blood the amounts dealt with are small The greatest cleanliness is therefore essential Different sets of pipettes and other apparatus should be kept for urine and blood estimations, it is advisable to have a separate pipette for each reagent, and special flasks, test tubes, etc reserved for each estimation Pipettes used for measuring blood should be washed out with distilled water immediately after use and at the end of the day washed out with alcohol and ether From time to time they should be washed with sulphuric acid bichromate mixture Blood and plasma should be discharged slowly from pipettes, otherwise an excessive amount will cling to the sides and the correct amount will not be delivered Small pipettes unless they have been tested by the National Physical Laboratory or one of its foreign equivalents are often grossly inaccurate 1 c.c. and 2 c.c. pipettes should be tested by running water from them into a weighed stoppered bottle and weighing again To test such pipettes with mercury is incorrect even if the mercury is pure, as they are not going to be used to measure mercury

* Dissolve 100 grains of potassium bichromate in 1 000 c.c. of water and add cautiously, 100 c.c. of strong sulphuric acid

Colorimetric methods When the colour of a solution is proportional to the concentration of a substance in the solution the concentration can be estimated by comparing the depth of colour of the solution with that of a solution of known concentration. To do this a colorimeter is necessary this consists essentially of an optical arrangement whereby two contiguous halves of a field are illuminated by light which has passed through known depths of two solutions.

The essentials are shown in Fig 8. The left half of the field is illuminated by light which has passed through the column of fluid A_1A_2 the right by fluid which has passed through the

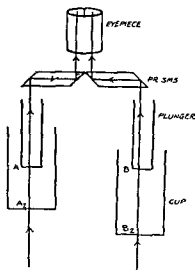


FIG 8 —Diagram of Color meter

column B_1B_2 . If the solutions in the two cups are coloured by the same colour and the columns A_1A_2 and B_1B_2 are so arranged that the two halves of the field match then

$$\frac{\text{Depth of colour in fluid on left}}{\text{Depth of colour in fluid on right}} = \frac{B_1B_2}{A_1A_2}$$

If the solutions contain a substance whose concentration is proportional to the depth of colour of the solution then

$$\frac{\text{Concentration on left}}{\text{Concentration on right}} = \frac{B_1B_2}{A_1A_2}$$

Scales are provided for reading the height of the columns A_1A_2 and B_1B_2 . It is usual to set the height of A_1A_2 in the standard

at some convenient figure and adjust the column B_1B_2 of the unknown until a match is obtained

Before use the colorimeter should be tested by filling both cups with the same solution and matching. The readings on both sides should be the same. If they are not the illumination may be unequal, or the scales may not give the heights of the columns of fluid correctly. Inequalities of illumination will be found to change by shifting the colorimeter relatively to the light source, colorimeters are usually fitted with arrangements for correcting the scales. Accurate readings can only be obtained when the depths of colour in the two fluids do not differ by more than some 40 per cent. It is therefore advisable to use amounts of the unknown which may be expected to give approximately the same depth of colour as the standard.

We have found the Bausch and Lomb pattern very satisfactory. Readings are best made by daylight. When artificial light must be used it is advisable to use a large hood in which the colorimeter and the worker's head can be inserted, illuminate with an artificial light that gives a uniform field and has a greenish coloured screen.

Colorimetric methods are, on the whole, quicker but less accurate than gravimetric and titration methods. A colorimeter is expensive, and were it not for the peculiar excellence of the Folin Wu blood sugar method and the absence of other methods for blood phosphate and some other estimations, we should hesitate to recommend its use. A cheap colorimeter, that of Myers, consists merely of two parallel graduated tubes which can be viewed against a ground glass plate, the unknown is diluted until it matches the standard. This simple form gives surprisingly good results, but cannot be used for accurate work.

Systems of blood analysis have been devised, in which the initial steps of the estimation of all the substances included are the same. The most conspicuous example is that of Folin. The advantages of such systems are obvious, but unfortunately none are suitable for all the substances, the estimation of which is of clinical value, we therefore give a series of methods without any attempt at such a system.

Sugar The glucose in blood is destroyed on standing, estimation should therefore be started without delay. If it is necessary to keep the blood it should be collected in tubes containing 30 mgm of potassium fluoride besides the usual

potassium oxalate the fluoride inhibits the breakdown of glucose

The only method of blood sugar estimation which can be recommended to those who are not expert chemists is that of Folin and Wu, to use this method properly however, a colorimeter is required. Those who wish to use a titration method are recommended to study one in a laboratory where it is used or at least to make sure of their technique by practising with blood to which a known amount of sugar has been added. The methods of Shaffer and Hartmann and of Hagedorn and Jensen are recommended.

Folin-Wu method

Required

2 c c pipettes

20 c c pipette graduated in c c

50 c c wide mouthed bottles with glass stoppers

Filter papers (Whatman No 1 9 c m diam)

Funnels 2 in diam

Small Petri dishes to cover the funnels

$\frac{1}{2}$ in test tubes

Boiling water bath

Folin's blood sugar tubes

Colorimeter

10 per cent sodium tungstate solution. The sodium tungstate should be readily soluble in water.

Two thirds normal sulphuric acid

Alkaline copper solution. Dissolve 40 grams of *anhydrous* sodium carbonate in 400 c c of water transfer to litre flask. Add 7.5 grams tartaric acid when this has dissolved add 4.5 grams finely powdered crystalline copper sulphate dissolve and make up to 1 litre.

Phosphomolybdic acid solution. In a litre beaker place 35 grams molybdic acid 5 grams sodium tungstate 200 c c of 10 per cent sodium hydroxide and 200 c c water. Boil vigorously to remove ammonia for about half an hour. Cool, dilute to 350 c c add 120 c c of phosphoric acid (specific gravity 1.75). Dilute to 500 c c.

Stock glucose solutions. Make a saturated solution of benzoic acid in a litre of water. Dissolve 1 gram of pure glucose (Kahlbaum) in saturated benzoic acid solution and make up to 100 c c. This stock glucose solution will keep indefinitely.

Standard I Measure 1 c c of the stock glucose solution into a 100 c c flask dilute to 100 c c with saturated benzoic acid solution

Standard II Make up in the same way with 2 c c of stock solution in 100 c c

Principle (a) The proteins of the blood are precipitated with tungstic acid and filtered off

(b) An alkaline copper solution is reduced by the reducing substances in the filtrate

(c) Phosphomolybdic acid added to the cuprous oxide so formed gives a strong blue colour

(d) The colour is compared in a colorimeter with that obtained by using a standard solution of glucose in place of the blood filtrate and the amount of glucose in the blood calculated from the relative depths of colour

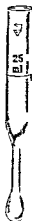


FIG. 9 Blood Sugar Tube

Procedure (a) Measure 2 c c of blood as soon as possible after withdrawal into a 50 c c bottle. Add 14 c c of distilled water and then 2 c c of 10 per cent sodium tungstate solution shaking gently after each add 2 c c of $\frac{2}{3}$ N

sulphuric acid shaking gently during the addition then stopper and shake violently the fluid should not froth. Stand fifteen minutes during which time the fluid should go brown.

Filter covering the funnel with a Petri dish the filtrate must be clear and colourless. Coloured filtrates usually result from insufficient acid and may be cleared by adding one drop of $\frac{2}{3}$ N sulphuric acid care should however be used not to add excessive amounts of acid as this will interfere with the next step. Citrates and excessive amounts of oxalates interfere with this precipitation.

(b) Measure 2 c c of filtrate into a blood sugar tube marked (X) into two blood sugar tubes marked (I) and (II) measure 2 c c of Standards I and II. To all three tubes add 2 c c of alkaline copper solution. Place in a boiling water bath for six minutes. The tubes are so made that the surface of the fluid comes into the narrow neck presenting a small surface to the air so that re-oxidation of the cuprous oxide formed is reduced to a minimum.

(c) Cool the tube in water for one minute and add to each

2 c c of phosphomolybdic acid solution When bubbles cease to appear dilute each to 20 c c Invert several times to mix

(d) Pour the fluid from (A) into one cup of the colorimeter into the other pour the fluid from (I) or (II) whichever appears to match (A) more closely Set the standard at 20 mm if a match is obtained with the unknown at x the concentration of sugar in the blood is

$$\frac{20}{10x} \text{ grams per 100 c c if Standard I was used for comparison}$$

$$\frac{40}{10x} \quad \text{II}$$

If Myers colorimeter is used when bubbles cease to appear in stage (c) pour the fluid in (A) into the left hand tube of the colorimeter wash (A) out with 2 c c of water three times adding these washings to the left hand tube mix Then pour fluid from tube (I) or (II) whichever more nearly matched (A) into the right hand tube of the colorimeter and wash in as before make this tube up to the mark 15 and mix well by inversion Then add water to the left hand tube mixing well after each addition until the tubes match If the level of the fluid in the left hand tube is x the sugar in the blood is—

$$\frac{x}{150} \text{ grams per 100 c c if Standard I was used}$$

$$\frac{2x}{150} \quad \text{II}$$

This instrument gives results which agree within 10 per cent with those obtained with the more delicate colorimeter It is therefore sufficiently accurate to give results of value in the diagnosis and treatment of diabetes mellitus

Micro method

When smaller quantities of blood are taken the above method may be still used

Required

0.2 c c pipette

Two graduated 1 c c pipettes

Stoppered 5 c c measuring cylinder

Other apparatus and reagents as above

Procedure Take 0.2 c c of blood and run it into about 2.5 c c of distilled water in the measuring cylinder washing the

pipette out by sucking water up and down. Add 0.4 c.c. of sodium tungstate with a graduated 1 c.c. pipette, and after standing add 0.4 c.c. of a $\frac{2}{3}$ N sulphuric acid. Make volume up to 4 c.c. with distilled water. Shake well and proceed as above. The filter papers used should be smaller and care taken to prevent evaporation by covering the funnel with a watch glass. The sugar in the blood is—

$\frac{40}{10x}$ grams per 100 c.c. if Standard I was used for comparison

$\frac{80}{10x}$ " " " II " " "

Ferricyanide method

Owing to the deep colour given, Fohn's ferricyanide method is better when the blood sugar is near the standard, but is inconvenient at higher levels owing to the necessity of correcting for the yellow colour of the ferricyanide.

Required

0.1 c.c. pipette

10 c.c.

4 c.c. pipettes

5 c.c. graduated pipettes

10 c.c. graduated pipette

20 c.c. centrifuge tubes

Boiling water bath

Fohn's blood sugar tubes

Dilute tungstic acid solution Transfer 10 c.c. of 10 per cent solution of sodium tungstate to a volumetric 500 c.c. flask dilute to about 400 c.c. Add with shaking

10 c.c. of $\frac{2}{3}$ N sulphuric acid and dilute to 500 c.c. Add a little toluol to prevent the growth of moulds.

Potassium ferricyanide solution Dissolve 1 gram of potassium ferricyanide (A.R.) in distilled water and dilute to 500 c.c. Keep in a brown bottle in the dark.

Sodium cyanide carbonate solution Weigh out about 2 grams of Merck's sodium cyanide and dissolve in distilled water to make approximately a 1 per cent solution. Transfer 8 grams of sodium carbonate to a 500 c.c. volumetric flask and dissolve in about 100 c.c. of distilled water. With a cylinder

add 150 c c of the cyanide solution Dilute to 500 c c and mix

Ferric iron solution Fill a litre cylinder to the mark Hang in this a gauze bag containing 20 grams of gum ghatti After twenty four hours filter the solution Dissolve 5 grams of ferric sulphate $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ and 75 c c of 85 per cent phosphoric acid in 100 c c of water with heating Cool and mix with the gum ghatti solution To destroy reducing substances present add 1 per cent potassium permanganate, at first 5 c c and later 3 c c at a time until the pink colour remains at least five to ten minutes

Stock glucose solution Dissolve 2 grams of benzoic acid in 500 c c of hot distilled water Wash 2 grams of glucose (Kahlbaum) with the benzoic acid solution into a litre volumetric flask Add water to about 900 c c, cool, dilute to 1,000 c c

Standard glucose solution I Place 0.5 gram benzoic acid in a 200 c c volumetric flask add 150 c c of water and 1 c c of the stock glucose solution Make up to 200 c c and add a few drops of toluol

Standard II As above with 2 c c of stock solution

The ferricyanide must be tested for ferrocyanide as follows Transfer 2 c c of a 1 per cent solution to a test tube add 3 c c of the ferric iron solution only the faintest blue colour should appear

Principle (a) The proteins are precipitated from the blood and are filtered off

(b) Potassium ferricyanide is reduced to ferrocyanide by the reducing substances in the filtrate

(c) A solution of a ferric salt is added and forms Prussian blue (ferric ferrocyanide) with the ferrocyanide This insoluble salt is held in colloidal solution by the gum ghatti

(d) The depth of colour is compared with that produced by a standard of glucose

Procedure (a) Collect 0.1 c c of blood and run into 10 c c of the dilute tungstic acid solution in a 20 c c centrifuge tube Wash the pipette out by sucking the acid up and down and stir well with the pipette Cover with a paper cap Centrifuge

(b) Measure 4 c c of the supernatant fluid, which should be water clear, into a blood sugar tube marked (X), into two other tubes marked (I) and (II) measure 4 c c of standards I and II To all these tubes add 1 c c of the potassium ferrocyanide

solution and 1 c c of cyanide carbonate solution. Heat for eight minutes in a boiling water bath.

(c) Cool the tubes in water for one minute and run 3 c c of ferric iron solution down the side of each tube. Shake and stand five minutes, make up to 20 c c and invert several times to mix.

(d) Compare in the colorimeter with the fluid from (I) or (II) which matches (X) most closely, setting the standard at 20 mm. If the reading of the unknown is over 30 or under 13 it is best to repeat the estimation using 2 c c of the standard or 2 c c of the unknown plus 2 c c of water. If the colours of the unknown and standard are seen before dilution to be very far apart repetition of the estimation may be avoided by including in the dilution the addition of 1 c c of ferricyanide solution to the tube whose contents are much more blue than the other. These adjustments have to be made because of the yellow colour of the unreduced ferricyanide. They can be rendered unnecessary by the use of a powerful artificial light covered by a specially prepared yellow screen made by soaking filter paper first in picric acid and then in paraffin solution.

If the standard is set at 20 mm and the reading of the unknown is x the sugar in the blood is—

$$\frac{20}{10x} \text{ grams per 100 c c if standard I was used}$$

$$\frac{40}{10x}$$

II

Acetone Bodies

An indication of the amount of acetone bodies in blood can be obtained by the nitroprusside reaction as follows —

Required Nitroprusside solution. Dissolve 30.5 grams of ammonium nitrate and 2.5 grams of sodium nitroprusside in water and make up to 100 c c. This reagent keeps for several months.

Procedure To 2 c c of the filtrate as obtained in the blood sugar method add 0.7 c c of the nitroprusside solution. Then add about 0.5 c c of strong ammonia solution and mix. A pink colour appearing in ten to twenty minutes and gradually deepening is evidence of an excess of acetone bodies. No attention should be paid to a pinkish colour which appears at once and fades in about five minutes.

Cholesterol

Required

2 c c pipette

10 c c , 5 c c , 1 c c and 0.1 c c pipettes

50 c c graduated flasks

10 c c stoppered graduated cylinders

Glass evaporating basins about 2 in diameter

Funnels, and small Petri dishes to cover them

Alcohol-ether mixture, containing three volumes of alcohol to one of ether

Chloroform

Acetic anhydride

Pure concentrated sulphuric acid

Standard cholesterol solution, 0.016 per cent in chloroform (5 c c contain 0.8 mgm) Dissolve 0.64 gram of cholesterol in chloroform and make up to 200 c c with chloroform Dilute 5 c c of this solution to 100 c c with chloroform to obtain the standard solution

Principle (a) The proteins of the blood are precipitated and the fatty substances are extracted by the alcohol-ether mixture

(b) The cholesterol is dissolved out with chloroform, which will not dissolve soaps, and the colour produced by adding acetic anhydride and sulphuric acid compared with that obtained from a standard solution of cholesterol It is essential that all glassware used should be quite dry

Procedure (a) Run 2 c c of blood or plasma, into a 50 c c graduated flask about two thirds full of alcohol ether mixture, shaking the flask gently meanwhile Hold the flask by hand in boiling water until the alcohol-ether mixture boils (do not stand the flask on a boiling water bath, as the liquid often bumps badly) Cool and make up to 50 c c Filter, covering the funnel with a Petri dish to check evaporation If the filtrate is kept in a well corked bottle it will keep indefinitely

(b) Evaporate 10 c c of the filtrate on a water bath Extract with 2 c c of chloroform and pour into a 10 c c graduated cylinder, which must be quite dry, wash out the dish twice with about 1 c c of chloroform and make the contents of the cylinder up to 5 c c, with chloroform Measure 5 c c of the standard solution of cholesterol into another cylinder To each cylinder add 1 c c of acetic anhydride and 0.1 c c of pure concentrated sulphuric acid, stopper and mix

Stand for fifteen minutes at 20° to 22° C in the same light as that by which the colorimeter readings will be made. Compare in the colorimeter with the standard at 20 mm. If the reading of the unknown is x , the blood (or plasma) contains $\frac{4}{x}$ grams of cholesterol per 100 c c.

To obtain accurate results the standard and unknown should not differ by more than 30 per cent.

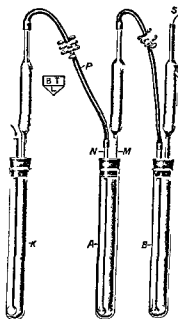


FIG 10 — Urea Apparatus

Blood Urea

Required

Coarsely ground soya bean or urease tablets

Pestle and mortar

2 c c and 10 c c pipettes

10 c c burette

Apparatus, as shown in diagram (Fig 10)

Jar of hot water, at about 50° C

0.6 per cent acid potassium phosphate

$\frac{N}{100}$ NaOH and $\frac{N}{100}$ HCl, made

up from $\frac{N}{10}$ solutions by diluting

25 c c to 250 c c with distilled water, and kept in hard glass

bottles. 10 c c of acid should from time to time be titrated with the soda, if they are not exactly equivalent a correction factor should be used, e.g., if 10 c c of HCl require 10.2 c c of NaOH the amount of NaOH used in subsequent titrations should be multiplied by $\frac{10.0}{10.2}$ to make it equivalent to the HCl.

Methyl red saturated solution in alcohol

Anhydrous potassium carbonate

Caprylic alcohol

Principle (a) The urea in the blood is converted into ammonium carbonate by the action of the enzyme of soya bean, which acts specifically on urea.

(b) The ammonia so formed is drawn off by a current of air and absorbed in a known volume of $\frac{N}{100}$ HCl

(c) The HCl left unneutralised is titrated with $\frac{N}{100}$ NaOH. The amount of HCl neutralised by ammonia is then given by difference from this the amount of ammonia given off and hence the urea in the blood are calculated.

Procedure (a) In tube A put 2 c c of blood add a knife point of soya bean or one tablet finely ground with water 2 c c of 0.6 per cent acid potassium phosphate and 2 drops of caprylic alcohol. Shake and place in the water at 50° C in the jar leave half an hour shaking occasionally the water in the jar need not be renewed. As the fluid is acid ammonia will not escape at this stage.

(b) Put 10 c c of $\frac{N}{100}$ HCl and 1 drop of methyl red solution in tube B and about 10 c c of dilute sulphuric acid in tube K. Attach as shown to water pump. Run a gentle current of air through. Remove stopper from tube A and drop in 4 grams of anhydrous potassium carbonate measured roughly in a test tube.

Replace stopper run current of air gently for five minutes then run as fast as possible without the fluids bubbling over leave one hour with the air current running.

(c) Detach from water pump (do not turn off the tap before detaching). Wash down the inlet tube of tube B inside and outside with distilled water and titrate the acid left with $\frac{N}{100}$ NaOH. If x c c of NaOH are used the urea in the blood equals $(10-x) \times 0.015$ gram per 100 c c.

The acid in tube K removes any ammonia in the incoming air.

The amount of acid used is sufficient if the blood urea is not above 0.150 per cent. If it is suspected that the blood urea is higher than this 1 c c of blood and 20 c c of acid should be used. If the methyl red turns yellow during stage (b) 10 c c more acid should be added at once a little ammonia will be lost but it will be possible to obtain a rough estimate of the blood urea which will indicate how much blood and acid to employ when the estimation is repeated.

No appreciable change takes place in the blood urea if the blood is kept twenty four hours

Fluoride interferes with the action of the soda bean and must not be used

Plasma Bicarbonate

The Alkali Tolerance test may be useful as a method of measuring the plasma bicarbonate. The reaction of the patient's urine is estimated roughly (p 322), usually, if acidosis is present, the urine will give a red colour with methyl red. 10 grams of sodium bicarbonate dissolved in 100 c.c. of water are then given every hour until the reaction of the urine becomes definitely more alkaline (e.g., instead of a red colour with methyl red gives orange or yellow). If g grams are required the plasma bicarbonate b is given approximately by the formula

$$(66 - b) = \frac{38g}{W},$$

where W is the weight of the patient in kilograms

For example if the weight of the patient is 50 kilograms and 30 grams of sodium bicarbonate are required the plasma bicarbonate is 43

Less correct but still useful results can be obtained if the alkali is continued until the urine turns alkaline to litmus

The method is not applicable in nephritis, as the kidneys cannot be trusted to respond to changes in the plasma

Normally 5 grams are sufficient to render the urine alkaline

Chlorides

Required

1 c.c. and 10 c.c. pipettes

10 c.c. burette

10 × 1 in. Pyrex tube

Iron alum powdered

$\frac{N}{50}$ silver nitrate solution Weigh out 3.398 grams of pure

silver nitrate dissolve in distilled water, add approximately 5 c.c. of pure nitric acid, and make up to 1 litre

$\frac{N}{50}$ Potassium thiocyanate solution Weigh out 2.0 grams of pure potassium thiocyanate using crystals as dry as possible

dissolve in distilled water and make up to 1 litre. Measure 10 c.c. of silver nitrate solution into a 10 × 1 in. tube, add a knife point of iron alum and titrate with the thiocyanate solution until a faint red colour appears. If y c.c. of thiocyanate solution are used, the factor for converting into its equivalent of silver solution is $\frac{10}{y}$.

Principle (a) Chloride is precipitated as silver chloride by the addition of a known amount of silver nitrate. At the same time the proteins are destroyed by heating with nitric acid. As silver nitrate is present in excess, no chlorine escapes.

(b) The silver nitrate remaining is titrated with thiocyanate; the amount precipitated can be calculated and hence the amount of chloride present.

Procedure (a) Measure 1 c.c. of plasma into a Pyrex test tube, add 10 c.c. of silver nitrate solution and 40 drops of nitric acid. Place in a boiling water bath and leave until the precipitate of protein has dissolved and that of silver chloride has formed a large grained deposit at the bottom of the tube. If whole blood is used, 2 c.c. of nitric acid should be used and the heating continued until the brown colour disappears.

(b) Cool and if the volume is under about 5 c.c., make it up to this with distilled water. Add a knife point of iron alum and titrate with thiocyanate until a faint red colour appears, which lasts on shaking.

If x c.c. of thiocyanate are required, $10 - \frac{10x}{y}$ c.c. of silver nitrate solution must have been precipitated by the chlorides in the plasma, so that 1 c.c. of plasma contains $\left(10 - \frac{10x}{y}\right) \times 0.00117$ gram of chloride reckoned as NaCl. That is, there are $\left(10 - \frac{10x}{y}\right) \times 0.00117 \times 100$ grams of chloride reckoned as NaCl per 100 c.c. of plasma.

Inorganic Phosphates

Required

2 c.c., 5 c.c. and 1 c.c. pipettes

10 c.c. graduated flasks

Ash free filter paper (Whatman No. 40, 5.5 cm. in diameter)

Funnels

Colorimeter

80 THE CHEMICAL EXAMINATION OF THE BLOOD

Standard phosphate solution Dissolve 0.4394 gram dry acid potassium phosphate and make up to 1 litre adding 1 c.c. of strong sulphuric acid to the water used in making up this is the stock solution of which 1 c.c. contains 0.1 mgm inorganic P. Dilute 20 c.c. of this solution to 200 c.c. this is the standard solution. 2 c.c. contain 0.025 mgm P.

Trichloroacetic acid 20 per cent

Molybdate solution Dissolve 20 grams ammonium molybdate in 300 c.c. of water add 75 c.c. concentrated sulphuric acid to 120 c.c. of water cool make up to 200 c.c. and add to the above solution

Hydroquinone solution Dissolve 0.5 gram hydroquinone in 100 c.c. of distilled water add 1 drop of pure sulphuric acid keep in a well stoppered bottle

Sulphite solution Dissolve 20 grams crystalline sodium sulphite in 100 c.c. of distilled water Make up fresh every week

Principle (a) The proteins are precipitated by trichloroacetic acid

(b) The phosphate present is converted into phosphomolybdate

(c) The phosphomolybdate is reduced to give a blue colour the depth of which is compared with that given by a standard phosphate solution

The phosphoric esters present in blood break down when the blood is kept if blood is used it must be precipitated at once. If plasma is used no harm will come from moderate delay after it has been separated

Procedure (a) Measure 2 c.c. of blood or plasma into a 10 c.c. flask add about 5 c.c. distilled water and gradually with shaking 2 c.c. of trichloroacetic acid solution make up to 10 c.c. with water and shake vigorously Stand at least 10 minutes and filter through an ash free paper

(b) Measure 5 c.c. of the filtrate into a 10 c.c. flask into another measure 2 c.c. of the standard and add 3 c.c. of water to each add 2 c.c. of molybdate solution mix

(c) To each add 1 c.c. of sulphite solution and 1 c.c. of hydroquinone solution mixing after each Make each up to 10 c.c. with water mix and stand half an hour

Compare in a colorimeter with the standard set at 20 mm. If the reading of the unknown is x then 100 c.c. of the blood or

plasma contains $\frac{20}{x} \times 0.0025$ gram inorganic phosphorus

This process is the reverse of the blood sugar method in which excess of phosphomolybdate is reduced by the substance to be estimated. It is therefore necessary to clean the cups and plungers of the colorimeter very thoroughly when passing from the one method to the other.

Calcium

Required

2 c c 5 c c and 10 c c pipettes

50 c c centrifuge tubes of greenish or pure white glass with conical ends

2 c c burette with glass stopcock

Filter papers

Filtered saturated solution of ammonium oxalate

$\frac{N}{100}$ potassium permanganate This is most conveniently

made by diluting a $\frac{N}{20}$ solution. Dissolve 1.581 grams of the purest potassium permanganate in distilled water and make up to 1 litre. Dilute 50 c c of this solution to 250 c c giving

approximately $\frac{N}{100}$ with this solution titrate 10 c c of $\frac{N}{100}$ sodium oxalate solution to which 1 c c pure H_2SO_4 has been added. If y c c are required the factor for converting a number of cubic centimetres of this solution to cubic centimetres of $\frac{N}{100}$ is $\frac{10}{y}$. The permanganate solutions should be kept in

amber bottles protected from dust. the bottles should not be changed as permanganate solutions keep better in old bottles.

Little change takes place in the $\frac{N}{100}$ solution in fourteen days but it should be standardised against sodium oxalate solution every week.

$\frac{N}{100}$ sodium oxalate solution. Dissolve 0.335 gram of oxalate in distilled water add 1 c c of pure sulphuric acid and make up to 500 c c. This solution will keep fourteen days in hot weather.

Saturated solution of calcium oxalate. To 10 c c of 1 per cent calcium chloride in a tube add 5 c c saturated ammonium oxalate solution, mix and stand three hours and centrifuge.

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Pour off the supernatant fluid, stir up with 40 c c of distilled water, centrifuge, and pour off again, repeat this four times. Wash the remaining precipitate of calcium oxalate into a flask, add 1,000 c c of water and 2 c c of strong ammonia solution, shake frequently, after three hours, filter through an ash free paper*. The filtrate should be tested by taking 20 c c, adding 0.5 c c strong H_2SO_4 and titrating with $\frac{N}{100}$ potassium permanganate, not more than 0.3 c c should be required.

2 N sulphuric acid

All glass apparatus used in this estimation must be washed first with acid and then with distilled water. The conical tubes must be cleaned with hot sulphuric acid bichromate mixture for one hour and washed well with distilled water each time they are used.

Principle (a) The calcium is precipitated as calcium oxalate.

(b) The precipitate is separated by centrifuging. As calcium oxalate is slightly soluble in water, a saturated solution of calcium oxalate is used for washing, in order to assist the solution of any protein which may contaminate the precipitate. ammonia is added to this solution.

(c) The precipitate of calcium oxalate is dissolved in sulphuric acid and titrated hot with $\frac{N}{100}$ potassium permanganate.

Procedure (a) Collect at least 6 c c of blood in a dry test tube, cork and stand until serum separates. Pipette off serum into a centrifuge tube, cover the tube with a cap and spin. Pipette off the clear serum and measure 2 c c into a conical centrifuge tube. Plasma from blood that has been prevented from clotting by the use of 1 mgm of heparin to 5 c c of blood is better than serum, as more can be obtained from a given amount of blood without delay in separation and risk of change in the calcium concentration, while standing over the corpuscles, is avoided. Add about 2 c c of distilled water and 5 c c of the ammonium oxalate solution, mix, cover with a cap and stand at least three hours.

(b) Centrifuge, pour off the supernatant fluid gently down a glass rod watching the precipitate to see that it is not disturbed, this can be best done if the tube is held over a

* The washed calcium oxalate should be kept and may be used again and again.

piece of black paper. If any precipitate shows signs of breaking away from the mass at the bottom, centrifuge again. Invert the tube on a piece of filter paper for two minutes then wipe round the inside of the upper half of the tube with filter paper to remove any remaining fluid. Run in 20 c.c. of saturated calcium oxalate solution from a fine pointed pipette first breaking up the precipitate and then washing the sides of the tube, replace cap and centrifuge. Pour off supernatant fluid, invert tube and wipe as before.

(c) Add 5 c.c. of 2 N H_2SO_4 stand the tube in water at about 80°C and when hot titrate with $\frac{\text{N}}{100}$ permanganate until the faintest shade of pink appears in the fluid and remains fifteen seconds after mixing. This is best seen by holding the tube over a piece of white paper and comparing with another tube containing water only. It is impossible to do this titration in ordinary artificial light.

A blank titration of 5 c.c. of hot 2 N H_2SO_4 should be made to determine how much permanganate is required to produce this faint pink colour (about 0.03 c.c.). If x c.c. of the $\frac{\text{N}}{100}$ permanganate are required for the titration of the precipitate from the serum (or plasma) and the blank is a c.c. then the amount of calcium in 100 c.c. of serum (or plasma) is—

$$\frac{1}{100} \times (x - a) \times \frac{10}{y} \text{ grams}$$

It is essential to clean the centrifuge tubes thoroughly otherwise the precipitate sticks to the sides and is lost when the fluid is poured off, caps are necessary to prevent dust falling into the centrifuge tubes a piece of paper held on with a rubber band is convenient. Occasionally the precipitate is contaminated with organic matter which does not dissolve when the sulphuric acid is added in this case the results are too high.

Bilirubin in Plasma

A slight excess of bile pigment in plasma (or serum) imparts a yellow colour, which is easily detected unless hæmolysis has occurred.

Icteric index Compare plasma or preferably serum with 0.01 per cent potassium bichromate in a colorimeter. The ratio of the reading of the standard to that of the plasma or

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serum is the icteric index. The average normal index is 3.6. Owing to the possible presence of other yellow substances such as carotin, the results are unsatisfactory.

Fouchet's test is a fairly delicate one.

Required Fouchet's reagent. Dissolve 5 grams of trichloroacetic acid in 20 c.c. of water and add 2 c.c. of 10 per cent ferric chloride solution.

Mix 3 drops of plasma with 3 drop of this reagent on a glass tile. If moderate excess of bilirubin is present a green colour develops reaching its maximum in twenty minutes.

A more delicate test, which distinguishes between the different kinds of bilirubin and can be used quantitatively is that of Van den Bergh.

Required (a) Sulphanilic acid solution. Dissolve 1 gram of sulphanilic acid in water with 10 c.c. of concentrated hydrochloric acid and make up to 1 litre.

(b) Sodium nitrite solution. Dissolve 0.5 gram of sodium nitrite in 100 c.c. of water. This solution will keep a week.

Before a test mix 20 c.c. of solution *a* with 0.75 c.c. of solution *b* to form diazo benzene sulphonie acid (Ehrlich's diazo reagent).

Centrifuge tubes

3 graduated 1 c.c. pipettes

Graduated 5 c.c. pipette

For quantitative estimation Stock iron alum solution. Dissolve 0.1508 gram of ammonium iron alum in water containing 50 c.c. of concentrated hydrochloric acid and make up to 100 c.c. This stock solution keeps indefinitely. From it make up the standard solution by measuring 10 c.c. into a 250 c.c. flask adding 25 c.c. of concentrated hydrochloric acid and making up to 250 c.c. This standard solution will keep one month.

Ammonium thiocyanate solution 10 per cent

Colorimeter

Principle Bilirubin gives a violet-coloured azo compound with diazo benzene sulphonie acid. If the diazo reagent is added directly to the plasma the colour is obtained when the bilirubin is in one state only (direct reaction). If the proteins are precipitated with alcohol the colour is obtained whatever the state of the bilirubin.

The colour obtained in this last method may be used for quantitative estimation of the total amount of bilirubin.

present, as a standard for comparison a solution of iron thiocyanate in ether is used

As changes occur on keeping the test should be done on the day that the blood is taken

Procedure To 1 c c of plasma in a centrifuge tube add 0.5 c c of diazo reagent. If a reddish violet colour appears within a minute the reaction is direct and immediate. If a colour appears and slowly deepens the reaction is delayed direct. After fifteen minutes add 2.5 c c of absolute alcohol and 1 c c of saturated ammonium sulphate, mixing after each. Centrifuge. If a colour was not obtained before the addition of alcohol and is now present in the supernatant fluid, the reaction is indirect. If a quantitative estimation is required pipette off the clear supernatant layer into one cup of the colorimeter and compare with an ethereal solution of iron thiocyanate prepared as follows —

Mix 3 c c of the standard iron alum solution in a stout test tube, with 3 c c of the thiocyanate solution, add 12 c c of ether, cork with a well fitting ordinary cork (not rubber) and extract the iron thiocyanate formed by inverting cautiously several times

Read at once with the standard at 10 mm. If the reading of the unknown is x , the plasma contains $\frac{10}{x} \times 2.0$ mgm of bilirubin per 100 c c

This method measures all the bilirubin in plasma in whatever form it is present. Any plasma giving a positive direct reaction will give a positive indirect reaction, but the reverse is not true

A change both in the form and total quantity of bilirubin takes place if the plasma is kept for a day at room temperature, but keeping for a day in the ice chest has no effect

CHEMICAL CHANGES IN HÆMOGLOBIN

Carboxyhaemoglobin in blood may be recognised by the following tests. Dilute the suspected blood and a sample of normal blood 100 times with distilled water. Add water to one or the other until the depth of colour in the two tubes is the same

(a) 1 Compare the two samples in two similar test tubes, if the suspected blood contains more than 20 per cent of its

hæmoglobin in the form of carboxyhæmoglobin it will be distinctly pinker than the normal blood. Saturate some of the normal diluted blood with coal gas in another tube, the relative hues of the three tubes will give some indication of the proportion of hæmoglobin in the form of carboxyhæmoglobin in the suspected blood.

(a) 2 Add to each tube a knife point of sodium hydro sulphite, the normal blood will turn purple (reduced hæmoglobin) the unreduced carboxyhæmoglobin will in contrast appear yellowish while the unknown will have an intermediate hue, according to the proportion of carboxy hæmoglobin it contains.

(a) 3 Compare the spectra of the three tubes. That of the normal blood will be one faint band in the yellow, that of the carboxyhæmoglobin two better defined bands in the yellow, if the suspected blood contains some 20 per cent of its hæmoglobin as carboxyhæmoglobin these two bands can be seen easily.

(b) To 10 c c of the two diluted bloods in two similar test tubes add one drop of freshly prepared 5 per cent potassium ferricyanide. Mix well at once. Both bloods fade to a clear yellow, that containing CO will have a more orange tint. The change is complete in about three minutes and remains stationary for about fifteen minutes. A difference can be detected when under 10 per cent of the hæmoglobin is in the form of carboxyhæmoglobin.

(c) Boil the two diluted bloods. The normal blood forms a brown coagulum at once, the coagulum from carboxy hæmoglobin is at first red, but changes to brown on further boiling. The usual description of this test, which leaves out this last stage is misleading.

Carboxyhæmoglobin in the blood, in amounts sufficient to cause symptoms can, therefore, be detected easily, at or shortly after the time of poisoning. If the patient lives the carbon monoxide is washed out of the blood and is reduced to undetectable amounts in under six hours, although the nervous symptoms may continue.

Nitric-oxide-hæmoglobin resembles carboxyhæmoglobin but may be distinguished by the fact that the red coagulum formed by the boiling test remains red on further boiling.

Methæmoglobin, if present in large amounts, imparts a brown colour to the blood. Dilute the suspected blood and a

sample of normal blood 1 in 100 with distilled water and saturate both with coal gas. If the suspected blood contains large amounts of methæmoglobin it will be less pink than the normal. Smaller amounts of methæmoglobin can only be detected by the discrepancy between the oxygen capacity as estimated by the usual methods and that to be expected from the hæmoglobin content estimated colorimetrically by a method in which the hæmoglobin is converted to methæmoglobin. This requires very accurate analysis.

Cyanhæmoglobin may be detected as follows. Dilute 1 c.c. of blood with 99 c.c. of water, add cautiously an equal volume of *neutral* hydrogen peroxide (40 vols). Hæmoglobin froths but becomes bright red, if cyanhæmoglobin is present the red has a brown shade depending on the proportions present. Cyanhæmoglobin alone turns brown, then yellow and then colourless.

Sedimentation rate

Required Tubes about 0.5 cm. in diameter containing 1 c.c. and graduated in millimetres. (Cutler's tubes.)

Procedure Collect oxalated blood in the usual way. Fill the tube to the zero mark with the aid of a syringe. Read at intervals for one hour. The height of the column of red blood corpuscles at any time is expressed as the percentage of the height of the total fluid.

At the end of an hour the tube should be centrifuged in order to obtain a measure of the corpuscular volume.

If the blood is not being taken at the same time for other purposes, it may be collected in a 1 c.c. syringe into which 0.1 c.c. of sterile 3 per cent sodium citrate has been drawn. The needle is then removed the blood and citrate well mixed by inverting with a finger over the opening of the syringe. The plunger is set at the 1 c.c. mark and the syringe clamped in a vertical position with the point up. If the plunger does not fit tightly the end to the piston is supported. The readings are taken as before. The syringe may be left twenty-four hours to allow complete settling to occur, in order to estimate the corpuscular volume.

Blood stains

Suspected blood stains may be examined by the benzidine reaction used for the detection of blood in faeces. This reaction

is not specific for blood. The *hæmochromogen* test, which is specific for blood, may be performed as follows. To a scraping of the stain on a slide add 2 drops of a solution containing—

10 per cent NaOH	.	.	.	3 c c
Pyridine	.	.	.	3 c c
Saturated solution of glucose in water	.	.	.	3 c c
Water	.	.	.	7 c c

If this mixture has not been kept at least three days it should be warmed, until bubbles appear, before using. Cover with a slip and examine. If blood pigment is present the colour changes from green brown through dark red to salmon pink, and characteristic elongated curved crystals of *hæmochromogen* can be seen, under the microscope, in from one to six minutes.

SECTION II.—PARASITOLOGY

CHAPTER VI

BACTERIA

Introductory In this section no attempt is made to cover the whole subject of parasitology and mention is made only of those organisms which the student is expected to be conversant with and the clinical pathologist commonly encounters. The nomenclature employed follows that of the Medical Research Council's System of Bacteriology which is, or should be, available for reference in all students' libraries.

The bacteria are classed primarily on the basis of their morphology and staining reactions and will be found to fall into groups of which the individual members resemble each other, not only in their laboratory characteristics, but also in the nature of the lesions which they produce. This division into groups has other justifications since it indicates to some extent the origin of bacterial species. Presumably at one period of the world's history all pathogenic bacteria were saprophytes, living upon their hosts without harming them and without producing highly specialised and deadly toxins. In each group of pathogenic bacteria can be found saprophytic prototypes, closely resembling the pathogenic members in morphological, staining and cultural characters. As examples may be given the saprophytic staphylococci found on the skin and in dust, the harmless Gram negative cocci of the throat, the diphtheroid bacteria and the acid fast hay and butter bacilli.

The acquirement of toxins by bacteria has not been directed solely against humanity. From the same non pathogenic prototype can often be traced organisms causing similar diseases in animals and which may or may not be pathogenic to man. The tubercle bacilli are distinctive for man, bovines, birds and fish, and although these bacteria closely resemble each other in so far as our comparatively crude methods permit investigation, each member has become distinct and established. The rapid multiplication of micro-organisms might lead us to

expect a rapid variation of species, but since bacteria multiply by the simple process of splitting in two the minimal opportunity for variation exists, and such variation as does take place is largely evolved out of minute alterations in the composition of the bacterial toxin. Consequently we should not expect to change human tubercle bacilli into the bovine or avian species by passing them through numerous members of the appropriate animals over intervals of a few years, and as a fact the change does not occur. The establishment of species has taken place by natural selection in the course of ages.

The most virulent of human bacteria are still capable of maintaining in the tissues a harmless saprophytic existence. The pneumococcus, the hæmolytic streptococcus, the coryne bacterium diphtheriæ in the buccal cavity, the typhoid bacillus in the gall bladder, the colon bacillus in the renal pelvis may, and often do, exist without affecting the host. The presence of the specific organism is not the only factor of infection nor is the isolation of bacteria from the body the whole of diagnosis.

TABLE I *The Gram-positive Cocci*

Produce extra cellular toxins hæmolytic and leucocidal	<i>Aureus</i> <i>Citræus</i> <i>Albus</i>	Staphylococci	Large opaque pigmented colonies General turbidity in broth Liquefy gelatin Clot milk Green fluorescence in neutral red broth Ferment majority of carbohydrates with out gas		
Capsulated (non hæmolytic)	<i>Mucosus</i>	Type 1 Type 2 Type 3 Group 4	Pneumococci	General turbidity in broth No growth on gelatin Clot milk Bile soluble	Translucent pinpoint colonies
<i>Viridans</i> <i>Fæcalis</i> <i>Enterococcus</i>	Hæmolytic Mouth Bowel Anaerobic	Streptococci	Granular deposit in broth Do not liquefy gelatin Milk acid no clot		

This group of organisms comprises the common pyogenic cocci which give rise to a number of morbid conditions, having in common a comparatively brief incubation period, an acute onset, the local production of pus, a usually rapid recovery,

and a tendency to leave the patient predisposed to future attacks

The Gram positive cocci include the staphylococci, the pneumococci, and the streptococci

Staphylococci (Plate VII) The staphylococci grow in groups or clusters, an arrangement best seen in films prepared from solid media and least evident in broth culture. In preparations made from pus the organisms are usually in pairs with occasional clumps, and are readily taken up by the phagocytes

On slope or plate cultures the staphylococci grow in large, round, opaque discrete colonies with clean cut edges. In broth they produce a general turbidity. Litmus milk is usually acidified and clotted. Gelatin is liquefied, and a green fluorescence is produced in neutral red broth. The majority of the litmus carbohydrate media are acidified, but no gas is produced

These organisms therefore as a group grow readily in all the usual media and produce active changes in them. The staphylococci are divided, according to the colour of the pigment they produce when growing on solid media, into *S aureus*, *S citreus*, *S albus*. The pigment, which is chemically related to carotin, is most obvious in a recently isolated strain, but may not appear until the culture has been exposed to sunlight for some hours. The power of pigment production may be lost in old cultures, and it is probable that there is little racial difference between the varieties of staphylococci obtained from human lesions. As a general rule, *S aureus* is the most virulent of the staphylococci and the most active in culture media. *S citreus* occupies an intermediate position and is comparatively seldom met with. *S albus* is usually the least virulent and the least active in culture media. White staphylococci are frequently obtained from the skin which are practically non pathogenic and very inactive in the various media, often producing no change in litmus milk

The staphylococci growing on agar or in broth rapidly produce diffusible toxins having hæmolytic, tissue necrosing and leucocidal actions. The effect of the toxin upon the white cells is first to clump and then to disintegrate them, and has probably an important bearing upon the formation of septic emboli. The toxin production of different strains varies both in amount and in kind. It is exceptional, however, to find

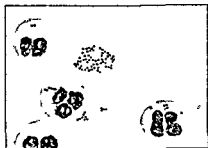
white staphylococci which give rise to powerful toxins or yellow strains devoid of all toxic properties

The most characteristic lesion produced by the staphylococci is the formation of a local abscess. The most virulent lesion is the acute suppurative epiphysitis or osteomyelitis of children, the causative organism being commonly *S. aureus* and less frequently a streptococcus, pneumococcus, or one of the other staphylococci. The staphylococci have a particular affinity for the lymphatic tissues and often give rise to metastatic abscesses in the lymph glands. The condition known as lymphangitis is practically always produced by *S. aureus*. Boils, carbuncles, and the suppurative lesions engrafted upon acne are among the local conditions caused by staphylococci. The organisms may further be spread from a local source by the blood stream and give rise to abscesses in the tissues and joints a condition known as pyæmia.

Numerous other cocci lead a saprophytic existence in the human body and may on occasion adopt a pathogenic rôle as secondary invaders of diseased tissues. The *micrococcus tetragenus* is an example of this class of organism and is distinguished from the pyogenic staphylococci by its habit of dividing in two planes at right angles to one another, and thus appearing in groups of four cocci. It grows in confluent white viscid colonies on agar and does not liquefy gelatin.

Pneumococcus (*Streptococcus lanceolatus*, *Diplococcus pneumoniae*) (Plate IX). The pneumococcus is commonly not rounded but shaped like the triangular blade of a lance, the bases of the triangles being opposed to each other in a pair. Not infrequently one member of the pair is lance shaped, the other round. Characteristically a diplococcus, short chains of four to six or even more may be found in pus and in liquid media. In a film of pus the almost invariable absence of phagocytosis is of considerable assistance in distinguishing this organism from the other Gram positive cocci. The pneumococci are capsulated the capsules being very obvious in pus films, appearing as clear spaces round the organisms, but more difficult to demonstrate in preparations made from cultures.

The appearance of Gram positive, extra-cellular, encapsulated, lance shaped diplococci in pus is very suggestive of the pneumococcus, but the diagnosis of the organism should never rest upon the film preparations alone, the cultural characters must be investigated also. The organism grows fairly readily



Staphylococci.
In pus from Abscess of Neck
(Carbol thionin)



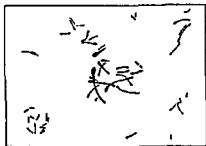
Streptococci.
In pus from a case of Cellulitis
(Carbol thionin)



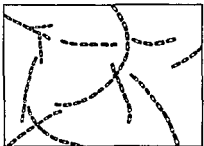
Gonococci.
In pus from Urethral Discharge
(Carbol thionin)



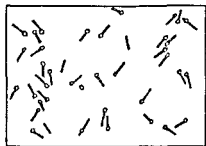
Tubercle Bacilli.
In Sputum
(Carbol Fuchsin and Methylene Blue)



Diphtheria Bacilli
Culture on blood serum
(Löffler's Methylene Blue)



Anthrax Bacilli.
Culture on Gelatin
(Carbol thionin)



Tetanus Bacilli
Anaerobic Culture on Agar
(Carbol Fuchsin and Methylene Blue)



Influenza Bacilli
In pus from Knee Joint
(Carbol Fuchsin)

on the ordinary media, but seldom so freely as the other members of this group. It grows well on blood agar or in milk. It does not grow on gelatin. On agar it produces minute round translucent colonies. Milk is acidified and usually clotted after two to three days. A general turbidity, together with a less obvious granular deposit, is produced in broth. Few only of the carbohydrate media are acidified, the most constant being dextrose, inulin and raffinose.

The pneumococci are not hæmolytic but on blood agar produce green colonies resembling those of *S. viridans* and in blood broth a purple discoloration. They have the property peculiar among bacteria of being soluble in bile and this specific test may be conducted by adding to an active broth culture one fifth volume of sterile filtered bile or 0.1 c.c. of a 10 per cent solution of pure sodium desoxycholate to 5 c.c. of the culture, which becomes clear in a few minutes.

The pneumococcus is highly virulent to mice and if inoculated subcutaneously the animal dies of a rapid septicæmia and films made from the heart blood or peritoneal fluid after death are found to be swarming with the capsulated bacteria.

The most characteristic lesion in man produced by the pneumococcus is lobar pneumonia, and the organism is abundant in the sputum and can usually be grown from the blood in the early stages of the disease. The pneumococcus is the commonest cause of empyema and may also produce an arthritis (usually of the large joints), peritonitis, a salpingitis and a meningitis. Arthritis and peritonitis may arise as sequels of lobar pneumonia or much more commonly independently of it. Pneumococcal meningitis is a somewhat rare affection, is usually secondary to otitis media, and runs a rapidly fatal course. The pneumococcus is a rare cause of conjunctivitis but is often associated with serpiginous ulcer of the cornea. A virulent form of infective endocarditis may be set up by the pneumococcus, sometimes as a sequel of lobar pneumonia but it is a rare complication. The organism may be found in the oral cavity of healthy persons and can frequently be isolated from the sputum in cases other than lobar pneumonia.

The pneumococci thus form a group of well defined organisms with distinctive morphological and cultural characters common to the group, and producing similar lesions in man and animals. They are subdivided mainly on serological differences, which

result from the chemical composition of the capsules. The capsular substances excreted by the pneumococci are carbohydrates which are protective to the bacteria and prevent them from being lysed by the body fluids or ingested by the phagocytes. Division is made into three main kinds known as Types I, II and III with a remainder composed of the other varieties which do not fall within any of the types and are classed together as Group IV.

Generally speaking proteins are antigenic (p. 185) and carbohydrates are not. The protein body of the pneumococcus is common to all the types and is antigenic, the isolated capsular substance is sharply specific for the different types and is antigenic only when adsorbed upon particles such as those of collodion or attached to protein such as that of the bacterial body. Consequently antisera can be produced by injecting an animal with the different types which will act specifically upon the capsular substance of the appropriate type causing it to swell up and disintegrate. The antisera will further effect specific agglutination of the bacteria. The methods employed for the typing of pneumococci are described subsequently (pp. 173 and 239). Of the three recognised types of pneumococci Type I is the commonest cause of lobar pneumonia and the least virulent and Type III the rarest and most virulent. Type III is also known as *streptococcus mucosus* owing to its mucoid growth in culture. It has also a great tendency to grow in chains and less disposition to the lance-shaped form. Its capsules are well marked and can be readily demonstrated in cultures. Group IV pneumococci are the varieties most frequently met with in the normal mouth and are also common causes of lobar pneumonia.

Antisera are made to the different types and being strictly type specific it is essential to type the infecting organism. Of these antisera Type I serum has the highest protective value. *Felton's serum* is a concentrated polyvalent serum widely used in the treatment of Types I and II pneumonia.

Streptococci (Plate VII) The streptococci comprise a considerable group of bacteria having the following characteristics in common. They grow in chains an arrangement best seen in liquid media. The number of cocci in a chain varies from 10 to over 100. In preparations made from pus short chains are usually numerous but the majority of the organisms may be in pairs and many of them are found within the phagocytes.

In cultures some of the cocci may show elongated bacillary forms. On solid media the streptococci grow in small round translucent "pin point" colonies. Broth shows a granular deposit on the sides of the tube with a few white granular colonies floating in an otherwise clear medium. Gelatin is not liquefied, and as a rule few of the carbohydrate media are acidified. The majority of the more pathogenic members acidify milk without clotting it.

The introduction of streptococci into the human body is followed by a brief incubation period, which may not exceed a few hours and a rapidly spreading inflammation. The bacteria tend to traverse lymphatic barriers and to enter the blood stream, often with fatal results. Recovery is usually rapid and immunity short lived.

No settled classification of the streptococci is yet possible either by serological methods or by their reactions in carbohydrate media but they are provisionally divided into hæmolytic and non hæmolytic the latter being further sub divided into mouth streptococci, bowel streptococci and anaerobic streptococci.

The hæmolytic streptococci have the property of breaking up red blood cells in culture. On blood agar plates the colonies show a colourless zone of hæmolysis around them and if a 5 per cent suspension of washed red cells in normal saline is added to a one day old culture in broth hæmolysis occurs in a few hours at 37° C. The production of hæmolysis in liquid media is the more definite test and is known as β hæmolysis. Among streptococci of other groups some have no hæmolytic action (γ type) others grow on blood agar with a zone of greenish discoloration due to the formation of an oxidation product of hæmoglobin. These organisms are known as *S. viridans*, and the form of hæmolysis as α hæmolysis. They are classed among the mouth streptococci. Evidence for the sub division of *S. hæmolyticus* into specific types is contradictory and there is much overlapping both in their fermentative actions upon the carbohydrates and in their agglutinins. There is considerable evidence that all the toxins of this group are immunologically identical. The names *S. pyogenes*, *S. erysipæ*, *S. latiss* and *S. scarlatina* are in common use and it is convenient to give a brief description of each though all may be identical.

S. pyogenes. The most characteristic lesions produced by this organism in man are the acute spreading inflammations of

the nature of cellulitis *S. pyogenes* may in addition cause acute inflammation in almost any part of the body, and the infections set up by it are usually of a serious nature. The lymph glands present a very feeble barrier to the spread of the organisms which not infrequently gain entrance into the general circulation and may be recovered from it in blood cultures. Puerperal septicæmia is a typical example of local infection by the *S. pyogenes* with a general dissemination in the blood stream. Infective endocarditis in the acute and virulent variety which follows a local infection is commonly produced by this organism. It is a not uncommon cause of pneumonia and in particular of the broncho pneumonias of children and after the pneumococcus is the most frequent organism found in empyemata. It is an important agent in the production of otitis media and of the purulent meningitis which may result.

S. erysipelatis is not agreed to be a specific organism. Erysipelas is an acute inflammation of the epidermis and cellulitis of the subcutaneous tissues and both are caused by hæmolytic streptococci. In animals erysipelas follows the intradermal injection of any hæmolytic streptococcus or even of pneumococci and the latter organism may produce an erysipelatous lesion in man. On the other hand there is clinical evidence that a case of erysipelas gives rise to similar cases and it has been claimed that the antisera to the toxin of *S. erysipelatis* produces blanching of the erysipelas and not the scarlatinal rash. The last observation has not however been confirmed.

S. scarlatinae. Important evidence in favour of a specific streptococcus of scarlet fever has been adduced by the Dicks. The organism can be isolated from the fauces in a high percentage of cases of scarlet fever and the cocci are agglutinated by the sera of convalescents. Cultures of the organism sprayed upon the fauces of volunteers have given rise to fever, angina and a scarlatiniform rash. A diffusible toxin is produced in broth culture and can be given intradermally to distinguish between those susceptible and those immune to the disease just as diphtheria toxin is used in the Schick test (p. 208). The toxin differs from the diphtheria toxin in being highly resistant to heat. The antitoxin prepared from the toxin is given in scarlet fever and good therapeutic results are claimed for it. The antitoxin has further the property of blanching the scarlatinal rash over an area round the site of injection in the same manner as does the serum of a convalescent case.

(Schultz Charlton reaction) Similar effects, however, can be obtained with antisera prepared to the toxins of hæmolytic streptococci from any source. There can be little doubt that hæmolytic streptococci are intimately concerned in the sore throat, the rash and many of the complications of scarlet fever, but it is still within reasonable speculation that the epidemic fever results from a virus infection, and the rash, the arthritis and the septic complications from a secondary streptococcal invasion. The solid immunity resulting from an attack of scarlet fever is sufficient ground for the belief that the association of hæmolytic streptococci with scarlet fever is not the whole problem.

The mouth streptococci include *S. viridans* and others of the γ type. Their cultural characters are similar to those of *S. hæmolyticus* but they have a tendency to grow in somewhat shorter chains and less luxuriantly. They are to be found almost constantly in the mouths of normal persons and commonly in the duodenum. They have low virulence for mice.

S. viridans is by far the commonest organism found in the blood and on the heart valves in cases of chronic infective endocarditis but its relationship to rheumatic fever and to chronic arthritis is much more problematical. Claims to have grown streptococci of the *viridans* and γ types from the blood and joints are numerous but the methods employed have been so elaborate that the possibility of contaminations cannot outweigh the results of the many observers who have obtained negative findings. *S. viridans* tends to multiply in the throat during epidemics of the common cold and plays the part of a secondary invader. It is probable that the pure virus stage of the cold is short lived and the chronic catarrh is due to the presence of these and other bacteria and vaccines prepared from the buccal flora often appear to have considerable prophylactic value. The streptococci may also play a part in the tonsillitis of rheumatic fever and in the form of nephritis which is frequently preceded by a sore throat. *S. viridans* may occasionally be obtained from the normal conjunctival sac and may produce a conjunctivitis. The more virulent form of conjunctivitis, associated with membrane formation, is usually due to *S. hæmolyticus*. In cases of infective endocarditis ordinary treatment by vaccine or serum therapy is useless. Attempts have been made to immunise a donor of the appro-

priate blood group with killed cultures of the organism grown from the patient's blood and to give transfusions of whole blood in amounts from 100 to 500 c.c. and some success has been claimed in a few cases.

The bowel streptococci include a group of long chained streptococci to which the name *S. enteritis* has been given a short chained group of the enterococci and the mannite fermenting group of Andrews and Horder, which appears to include members of both the other groups and which they class as *S. faecalis*. The enterococcus is the most distinctive of the bowel streptococci. In appearance it is a lanceolate diplococcus resembling and frequently mistaken for the pneumococcus but it is not bile soluble and is an active fermenter of the carbohydrates. Litmus milk is acidified, clotted and partly decolorised. In man the enterococcus is commonly obtained from the urinary tract and rarely in pure culture from cases of plevitis and cystitis. It exceptionally invades the blood stream and on the whole is an organism of low pathogenicity.

Anaerobic streptococci. Strict anaerobes are rare among the streptococci but are occasionally met with in the vagina and on other mucous surfaces. The group comprises a number of gas forming bacteria to which the names of *M. faecalis* and *S. putridus* have been given. The pathogenicity of the group is very low to animals and apparently to man also.

TABLE II

The Gram negative Cocci

Ferments glucose and not maltose	{	Gonococcus	{	Minute delicate colonies slow growth
Ferments glucose and maltose		Meningococcus		No growth on agar
Pigmented growth		Flavus		
Ferments no carbohydrate	{	Catarrhalis	}	Larger colonies grow on agar

The Gram negative cocci include two important pathogenic members the gonococcus and the meningococcus and a number of saprophytic bacteria which are mainly of significance because owing to their habitat staining and cultural reactions they may lead to errors of diagnosis. All the organisms of the group show a marked preference for certain special localities namely the urethra, vagina, meninges, conjunctival sac, nares and

pharynx In the United States the bacteria of this group are classed as the *Neisseria*

Gonococcus (Plate VII) The gonococcus is characteristically a diplococcus in which the individual members of each pair have the opposed surfaces flattened In a film of pus the majority of the cocci are found within the phagocytes, and a typical field shows large numbers of empty cells, together with one or two only of the polymorphonuclear or epithelial cells crowded with diplococci A film of pus in which the comparatively few cells which are phagocytic are distended with flattened Gram negative diplococci regularly disposed in the cells, particularly if obtained from a urethral or cervical discharge is sufficiently characteristic to justify the diagnosis of a gonorrhœal infection It must be remembered, however, that errors in the technique of using Gram's stain are common, and that an intracellular diplococcus is by no means necessarily a gonococcus It is unfortunate that owing to the difficulty of isolating the gonococcus in culture, the diagnosis has often to be made from film preparations, and in cases of any doubt the beginner should be far more cautious than is customary in expressing an opinion

The gonococcus will not grow at all on the ordinary media, but requires some medium containing the essential elements of blood serum A simple medium upon which to grow the organism is an agar slope over which has recently been smeared with a platinum wire a drop of sterile blood obtained by pricking the thumb A good medium for general purposes is the serum agar medium described on p 249 On this medium the gonococcus grows well, but somewhat slowly the small translucent 'pin point' colonies taking 24 hours or longer to reach maturity A more satisfactory but more complicated medium, is described on page 251 It is important that the culture media employed should be moist and preferably with visible water of condensation in the tubes There should be the minimum of delay between inoculating the cultures and incubating them The gonococcus very constantly produces acid in glucose and not in maltose media, and these reactions are best tested for in solid media to which the carbohydrates and a suitable indicator have been added The gonococcus rapidly dies out in culture tubes unless frequent subcultures are made

The usual infections produced by the gonococcus are intra-genital—that is to say, a urethritis in the male and a urethritis,

or more commonly an inflammation of the vagina and cervix uteri, in the female (see also p. 231). The diagnosis of the nature of an acute urethritis in the male is easy, the diagnosis of gonorrhœa in the adult female requires a special examination. A swab taken at random from the vaginal secretion is quite useless. Films made in this way, even from the normal vagina swarm with bacilli, cocci, and other organisms, so that it is a profitless labour to search through them for occasional gonococci. The films should be made from the interior of the urethra and, after the passage of a speculum from the cervical canal care being taken not to touch the walls of the vagina. The gonococcus is the commonest cause of pyosalpinx, but at the time of operation the organism has frequently died out and cannot be demonstrated in the pus. The infective vaginitis of little girls differs in some clinical respects from the gonorrhœa of adults in that it is extremely contagious and may spread through a children's ward. It has an unduly long incubation period and is very exceptionally followed by the ordinary sequelæ such as arthritis. The vaginal discharge of these cases contains large numbers of gonococci of typical appearance.

Among the complications of gonorrhœa in which the gonococcus may be detected are conjunctivitis (including the most frequent variety of ophthalmia neonatorum), arthritis and rarely a general septicæmia, with or without an infective endocarditis.

Meningococcus (Plate IX.) The meningococcus in films of pus obtained from the cerebro spinal fluid appears as a diplococcus. Some of the organisms are extra-cellular, but the majority may be within the phagocytes. The cocci are usually rounded but occasional pairs with flattened opposed surfaces may be seen and rarely a polymorphonuclear cell may be found distended with such pairs, the organisms thus closely resembling the gonococcus in appearance. Fortunately it is practically never required to distinguish between the two organisms, owing to the diverse situations in which they are found. Cultural differences are neither great nor absolutely constant. The meningococcus will often grow on ordinary agar in primary culture but the blood-containing media necessary for the gonococcus are advisable and almost essential for the meningococcus. In the carbohydrate media the meningococcus ferments both glucose and maltose, but these reactions are not

absolutely constant for all strains. The distinction on serological grounds is also inconclusive owing to cross agglutination between the two organisms and the difficulty in getting polyvalent sera against all types of either bacterium. The meningococcus like the gonococcus tends to die out in cultures and is best kept on Dorset's egg medium.

It appears from agglutination tests that more than one variety of meningococcus exists and by this means four types have been differentiated.

The meningococcus is the causative organism of cerebrospinal meningitis, both in its epidemic and sporadic form, and can nearly always be obtained in pure culture from the cerebrospinal fluid. During an epidemic the organism can frequently be grown from the blood in the early stages of the attack and from the hæmorrhagic skin lesions. The meningococcus gains entry from the pharynx and 'carriers' are responsible for the maintenance of the infection. The recognition of 'carriers' is difficult owing to the prevalence of other Gram negative cocci in the throat. Cultures of the pharynx should be taken with a West's swab (p. 401) to prevent contamination with the saliva which has a bactericidal action upon the meningococci, and plated on a serum agar or hydrocele agar medium. The small, transparent colourless colonies with sharply defined margins are picked off for further examination which must include agglutination reactions. For the agglutination test Fildes' method is advisable owing to the number of strains encountered. By this method a polyvalent serum is absorbed by the unknown strain and then tested against type cultures to determine which, if any, type agglutinin has been removed.

Micrococcus flavus is an apparently non pathogenic organism which has much cultural resemblance to the meningococcus but differs from it by producing a pale yellow pigment in primary culture and in its agglutinating properties. Other and more deeply pigmented Gram negative cocci are met with in the throat, but owing to their chromogenic properties should not be mistaken for meningococci.

Micrococcus catarrhalis. This coccus is the least important member of the group, and may be regarded as a more or less normal inhabitant of the nose and throat. In film preparations it appears as a fairly large rounded diplococcus, and is often found within the phagocytes. It grows feebly on agar in small colonies when first isolated, but more abundantly in subcultures.

The colonies are more opaque than those of the meningococci and unlike them emulsify badly in saline. No acidification of the carbohydrate media is produced.

TABLE III
The Acid fast Bacilli

More lethal to guinea pigs than to rabbits	Human type Bovine type Avian type	Tubercle bacilli	{ Slow & sly growth on glycerine and egg media
Lethal to rabbits and guinea pigs			
Not lethal to rabbits and guinea pigs			
		Lepra bacillus	{ Partially cultivated
Produces disease in cattle		John's bacillus	{ Grows on media to which other acid fast bacilli or their products have been added
Non pathogenic	Smegma bacillus Moeller's Timothy grass bacillus Rabinowitch butter bacillus		{ Grow with relative rapidity and on simple media

This important group of organisms belongs to the genus *Mycobacterium* and includes a number of bacteria pathogenic to man and animals and resembling each other in their need for special culture media and their slow rate of growth. The lesions produced are alike in their nodular or tubercular characters and in their chronicity. There are types of acid fast bacilli pathogenic for man, beasts, birds, reptiles and fish, and in addition numerous saprophytic organisms widely spread in nature. The bacilli have a tendency to produce long thread-like beaded and even branching forms and are no doubt fairly closely related to the streptothricæ, some of which are acid fast. The term 'acid fast' is used to indicate a staining reaction due to fatty and waxy substances incorporated in the bacilli and separable from them after prolonged extraction by ether or other fat solvents. These substances being resistant to ordinary dyes the bacteria are not coloured by simple staining processes but after staining with hot carbol fuchsin they are coloured red and will then resist decolorisation by mineral acids. Some members of the group are more easily decolorised by acid than others and some are decolorised by alcohol while others are not. The acid fast bacilli are all Gram positive.

M. tuberculosis (tubercle bacilli) (Plate VII). The detection of tubercle bacilli in preparations made from sputum, urine, or

other sources is described in subsequent chapters. Stained by the Ziehl Neelsen method (p. 217) the bacilli appear as thin curved and beaded red rods and are mainly extra cellular. They produce toxins which are intimately bound up with the bodies of the bacteria and which can be extracted by various processes to form the different tuberculins (p. 202). The tubercle bacilli are not generally believed to form spores but there is evidence of the possibility of an ultra microscopic stage of these, and conceivably other bacteria. Filtrates of tuberculous pus or of young cultures of tubercle bacilli in which bacteria can neither be seen nor demonstrated in culture have been found by some observers to set up an adenitis in distant lymph glands after a long interval of time. Acid fast bacilli have been recovered from these glands and after further passage or cultivation have yielded typical tubercle bacilli. Similar filtrates have produced tuberculosis in the guinea pig foetus without affecting the dam. Evidence of a filterable phase was first made by Fontes in 1910 and has recently been amplified and categorically confirmed by Calmette. The examination of similar filtrates by many other observers has however, yielded entirely negative results.

The *human tubercle bacillus* is by far the most important of this group of organisms and is responsible for the great majority of all human tuberculous lesions including nearly all cases of pulmonary tuberculosis. The human tubercle bacillus will grow on blood serum or on media to which glycerin has been added such as glycerin agar or glycerin broth as well as on inspissated and liquid egg media. Growth on solid media is very slow and only a slight granularity is seen in 10 days later the growth becomes wrinkled greyish and scaly. The human type grows better on glycerin-containing media than the bovine type. Old cultures on potato become deeply pigmented and of a reddish brown colour. Inoculated into animals the bacillus is found to be highly virulent to guinea pigs but to have comparatively little virulence for rabbits and calves.

The *bovine tubercle bacillus* is responsible for a proportion of the glandular and arthritic tuberculous affections particularly in children and much less frequently for pulmonary tuberculosis. Owing to the extreme frequency of tuberculosis among cows and the common presence of bovine bacilli in milk this source of infection for the human subject is no doubt a wide

one at the same time the typical human infection is pulmonary tuberculosis and the spread of tuberculosis in its most important aspect is by the human sputum. The bovine bacillus on serum grows more slowly than the human bacillus taking from 2 to 3 weeks to produce a thin greyish film which is neither wrinkled nor pigmented. It is highly pathogenic to calves and rabbits.

A bovine strain of tubercle bacilli has been cultivated by Calmette and Guérin in a bile medium for many years. It has remained acid fast but has lost its pathogenic properties for man and animals. It is claimed that the bacilli are still antigenic and injected into animals will convert a negative tuberculin reaction into a positive one. The strain is known as the BCG (Bacille Calmette Guérin) and has been widely used on the Continent in the prophylactic vaccination of children against tuberculosis.

The avian tubercle bacillus appears to be practically non-pathogenic to man and since tuberculosis is rare among birds in the wild state though common among those kept in captivity the bacillus can scarcely be regarded as a serious factor in the spread of human tuberculosis. The avian bacillus is readily recognised by inoculation experiments since it is highly virulent to pigeons but in guinea pigs produces only a local lesion.

The avian bacillus grows more rapidly than the human and in glycerine broth forms a granular deposit at the bottom of the flask in contrast to the human type which grows in a wrinkled pellicle on the surface.

The bacillus of fish tuberculosis does not grow at body temperature and is non-pathogenic to mammals.

Johnes's bacillus is another acid fast bacterium which produces a chronic inflammation of the intestinal mucous membrane of cattle. The organism will not grow on the ordinary media unless other members of the acid fast group or their products have been added.

The non-pathogenic acid fast bacilli embrace a number of organisms which differ from the pathogenic bacilli in their far more rapid growth on artificial media. They are practically non-pathogenic to animals and on inoculation into the highly susceptible guinea pig give rise to a small local lesion only. The group includes Rabinowitsch's butter bacillus, Moeller's timothy grass bacillus and a variety of similar organisms.

found in dust, manure, and other substances. These bacilli rarely cause confusion in human pathology, but owing to their occasional presence in tap water, in glassware as received from the manufacturer, and in chemical reagents including absolute alcohol, the finding of scanty acid fast bacilli in any specimen should be carefully controlled.

The smegma bacillus This organism is considered separately because of its human distribution and the liability to confusion with the virulent tubercle bacillus. The smegma bacillus is found in the genital secretions, and in film preparations appears as a shorter stouter, and less beaded rod than the tubercle bacillus. The smegma bacillus can be usually differentiated by its staining reactions since after treatment with 25 per cent acid it is decolorised by immersion in methylated spirit for 2 minutes. Some strains of smegma bacilli are, however, both acid and alcohol fast. The smegma bacillus grows luxuriantly on serum and glycerin agar media.

For the purposes of clinical pathology, the demonstration of bacilli which are strongly acid- and alcohol fast in the human tissues is sufficient for the diagnosis of a tuberculous lesion. The only organism likely to be confounded with the human tubercle bacillus is the bovine bacillus, and for practical purposes the two may be considered as identical.

In tuberculosis of the lung and of the urinary tract the bacilli are commonly found in large numbers in the sputum and urine. In the pus obtained from tuberculous sinuses and abscesses or from tuberculous joints the bacilli are almost always extremely scanty. The same is true of tuberculous body fluids, whether peritoneal, pleural, or cerebro spinal, also of the skin tuberculides. In the faeces the bacilli may be present in large numbers in cases of tuberculous enteritis and in very small numbers in pulmonary tuberculosis. In young children who swallow the sputum, tubercle bacilli may be recovered from the stomach by lavage. In all situations, other than the urine or the sputum it is necessary to adopt special processes for the demonstration of the organisms, and these will be described in a subsequent chapter.

M. lepræ (Hansen's bacillus) The leprosy bacillus closely resembles in appearance the human tubercle bacillus, but the following distinctions can be made out. The bacilli are very numerous in any leprosy lesion, whether in the pus from a breaking down focus, or from a sinus, or in microscopic sections

of lepra nodules. Many of the bacilli are seen within the cells, and certain elongated cells are found containing a number of bacilli arranged like a bundle of cheroots. Further, the lepra bacillus is not as a rule so acid fast as the tubercle bacillus, and may often be decolorised by 25 per cent sulphuric or nitric acids, retaining the stain only when treated with acids of half this strength. The lepra bacillus does not grow on the media usually employed for the tubercle bacillus, and until recently had never been cultivated.

Cases of leprosy are extremely rare in this country, and all are imported. The diagnosis can in nearly every case be confirmed by making film preparations of the nasal secretion. The majority of leprosy individuals have a chronic crusted nasal discharge containing the bacilli in large numbers, so that it is rarely necessary to excise a suspected nodule for microscopic examination.

In spite of the prevalence of the bacilli in the nasal secretion the disease appears to have very little direct infectivity and its mode of spread is still obscure.

TABLE IV *The Gram positive (non-acid fast) Bacilli*

Aerobes	Coryne bacteria	{	Diphtheria—toxin producing ferments glucose		
			Diphtheroids—ferment sac charose		
Aerobes spore - bearing, liquefy gelatin	Bacilli	{	Anthrax—non motile, capsu lated		
			Subtilis—motile non capsu lated		
Spore bearing toxin pro ducing anaerobes	Clostridia,	{	Tetanus	Motility +	Liq of gelatin. +
			Botulinus	+	+
			Welchii	—	+
			Edematiens	+	—
			Septique	+	+
			Chauvoci	+	+

This important group of Gram positive bacilli includes two classes of aerobes and one of strict anaerobes. Of the aerobic bacteria diphtheria and the diphtheroid bacilli belong to the genus *Corynebacterium*, anthrax and subtilis to the genus *Bacillus*. The anaerobes are of the genus *Clostridium*.

Corynebacterium diphtheriæ (*Klebs Löffler bacillus*) (Plate

VIII) The appearance of the diphtheria bacillus in films prepared from cultures is very characteristic but it is rarely possible to identify the organism in films made directly from the swab. The bacilli are arranged in small groups and the members of each group have a peculiar angular relation to each other so that the groups resemble the fingers of the hands crossed or a Chinese character. The individual bacilli are thin curved and beaded staining alternately in light and dark areas. They are non motile. Some bacilli may show bulbous or racquet shaped extremities and these so called involution forms are common in cultures more than 48 hours old. The bacilli are readily identified from their size shape beading and arrangement after staining with Löffler's methylene blue. Neisser's stain (p 233) is an additional aid in distinguishing the diphtheria bacilli since the metachromatic granules are well shown by it and it is exceptional to meet with diphtheroid bacilli positive to Neisser's method. The organisms grow well on the ordinary media but it is preferable to make the primary culture on Löffler's serum since the diphtheria bacillus tends to outgrow the other throat bacteria in the first 12 hours and the characteristic appearances are more constantly developed. On solid media the bacilli grow in colonies of the streptococcal type but they are slightly more heaped up and opaque. A general turbidity is produced in broth and with good toxin producing strains a pellicle is formed on the surface. The filtrate of a 10 day broth culture contains the powerful extra cellular toxin sometimes in such concentration that 0.001 c.c. will kill a guinea pig. Of the carbohydrates the diphtheria bacillus constantly acidifies glucose and galactose but never saccharose no gas is produced. Gelatin is not liquefied.

If a suspension of the bacilli is introduced into the leg of a guinea pig death results in about 36 hours and *post mortem* a small greyish necrotic membrane is found at the seat of inoculation. Hæmorrhages are present in the supra renals and the cardiac muscle on microscopical examination is found very extensively affected by a fine fatty degeneration. If a second guinea pig is inoculated with the bacilli or their toxins together with anti diphtheritic serum no ill effects result.

These procedures constitute a form of virulence test such as is essential for the certain recognition of the toxin producing diphtheria bacillus.

The diphtheria bacillus is the pathogenic type of a large

number of bacilli the non pathogenic members being known as diphtheroid bacilli

Diphtheroid bacilli are very widely distributed in the human body and it is essential to have some knowledge of them owing to the extremely close resemblance they bear to the diphtheria bacillus itself. The diphtheroid bacilli include those which can be distinguished from the Klebs Löffler bacillus on morphological grounds those which cannot be so distinguished but which have cultural differences and those which are both morphologically and culturally identical.

Hofmann's bacillus can be distinguished on morphological grounds by its appearance in film preparations made from a culture. The bacilli are distributed in groups the members of which have a tendency to a parallel rather than an angular arrangement. They are short and rarely curved and are not truly beaded but stain deeply at each end displaying a pale band across the middle.

They are more strongly Gram positive than the diphtheria bacillus and are negative to Neisser's stain.

C. xerosis is the name given to a diphtheroid organism commonly met with in the conjunctival sacs. It closely resembles *C. diphtheriae* in appearance but rarely gives Neisser's reaction. It grows badly on agar and in broth leaves the medium clear forming a granular deposit at the bottom. It ferments saccharose.

The *acne bacillus* in film preparations resembles the diphtheria bacillus in its grouping but is smaller and is not beaded being in appearance not unlike Hofmann's bacillus. It grows with difficulty on the ordinary media except in subculture and the first culture is best planted on oleic acid glycerin agar and grown under anaerobic conditions. The acne bacillus is obtained in considerable numbers from the depths of acne comedones. When suppuration occurs a staphylococcus is usually present in addition.

The causal relationship of the bacillus to acne is not certainly established and the treatment of acne by vaccines made from this organism is of doubtful value.

Other bacilli which are frequently isolated from the urethra and less often from any of the above situations resemble the diphtheria bacillus exactly both in their morphology and in their cultural characters. They differ however in one very important respect—they produce no toxin and are conse-

quently non pathogenic to guinea pigs. The relationship of the non toxin producing bacilli to the pathogenic diphtheria bacillus is uncertain, and the possibility that an organism which produces no toxin in culture and is non lethal to animals might yet be capable of re acquiring properties temporarily lost, cannot be dismissed, but in practice it appears to be justifiable to regard a diphtheria like bacillus which is non lethal to the guinea pig as harmless to human beings.

If a bacillus is obtained from the throat or larynx of a person whose clinical condition suggests diphtheria, and if it is found to resemble the diphtheria bacillus in appearance, the patient should be isolated and given anti toxin. In the case of nasal diphtheria the full cultural and morphological characters of the bacilli should be ascertained, since diphtheroid bacilli are common in this situation, and greater attention should be paid to the clinical condition than to the bacteriological findings. In patients convalescent from diphtheria the persistence of bacilli is usually due either to the presence of a small tonsillar lesion requiring treatment or to the appearance of diphtheroid organisms. In such cases as well as in suspected diphtheritic lesions in unusual places, such as the conjunctiva, surface wounds, the vagina, etc., the morphological and cultural characters of the bacillus are insufficient for diagnosis. In all cases of doubt or of exceptional importance (such as a sporadic case of diphtheria in a school) the bacilli must be isolated and animal inoculations performed.

The routine bacteriological examination of the throat is therefore of great assistance in suspected cases of diphtheria, but the results should be accepted with reserve in other situations, and should not be regarded as proven until a guinea pig has been inoculated.

The successful isolation of the bacilli from the throat is largely dependent upon the care with which the culture is taken. The necessary apparatus consists of a blood serum culture tube and a second tube containing a straight piece of stout copper wire, around one end of which has been firmly twisted a piece of absorbent wool. Wool and wire must be sterile. In the case of a refractory child have the arms, legs, and body wound in a blanket and the child's head held by a nurse. Choose a good light, depress the tongue with a spatula, pass the cotton-wool swab on to the membrane and rub it firmly into the lesion. Withdraw without touching the tongue

or cheeks Rub the swab over the surface of the serum and replace in its own tube without burning it Incubate for 12 hours Examine films stained with Löffler's methylene blue from both swab and medium A direct examination of the swab, after making the culture, may give information about the presence of other bacteria, for example, the prevalence of Vincent's organisms, but can only be tentative in the diagnosis of diphtheria

B anthracis (Plate VII) Anthrax infections in man are extremely rare and occur in two main forms The more usual variety is a local infection of the skin, arising among hide porters and workers, and known as the malignant pustule With treatment the prognosis in this form is good Treatment by serum has largely replaced that of excision, and the serum most commonly used is Selavo's, which is obtained by the immunisation of asses to the bacillus The mortality of serum treated cases is under 5 per cent The other variety is known as wool sorters' disease, and in this the local infection is in a bronchus extension taking place through the bronchial glands This form is almost invariably fatal In films made from the clear fluid of the bleb of a malignant pustule occasional pus cells are found, and large numbers of long, stout bacilli, with sharply cut ends, are seen The majority of the bacilli are arranged in long chains, each chain being contained within a delicate capsule In films made from old cultures the great majority of the bacilli are seen to contain central spores, not infrequently spores only are found and no bacilli The bacillus grows readily on all the ordinary culture media, the most characteristic growths being found on agar plates and in gelatin stabs On agar plates the colonies examined with a magnifying glass appear like wreathed coils of hair, an appearance produced by the long twisted strands of bacilli In gelatin stab cultures growth occurs along the line of the stab and branches out from this in spokes radiating into the medium The spikes nearest the surface are the longest, so that the growth looks like an inverted fir tree *Laquefaction* of the gelatin begins at the surface on about the second day of the growth

The organism is non motile, an important point of distinction from non pathogenic spore bearing bacilli Toxin formation by the anthrax bacillus is possible but not proven

The recognition of the anthrax bacillus in the fluid from a

malignant pustule is easy, but in sputum, and particularly in suspected articles such as shaving brushes diagnosis is difficult because of the common distribution of non pathogenic bacteria of similar appearance. A test for pathogenicity should always be made by injecting a culture into the leg of a guinea pig. This animal is highly susceptible and dies in about 3 days with a general septicæmia and a gelatinous œdema, spreading from the site of inoculation.

B. subtilis (hay bacillus) is one of a group of bacilli whose chief importance in clinical pathology is their frequent occurrence as contaminants in culture media. The bacilli are sluggishly motile and have central spores but no capsules. They liquefy gelatin slowly, render litmus milk alkaline and form a tough pellicle on the surface of broth tubes.

The clostridia form a group of anaerobic spore bearing bacilli with powerful extra cellular toxins. All are faecal contaminants of the soil.

The tetanus bacillus (Plate VII). Tetanus bacilli when growing in the tissues commonly have few, if any, spore bearing forms. On artificial media the majority of the bacilli contain spores. The bacillus under these conditions is comparatively slender and the delicate rounded spore is situated at one extremity. Other spores are seen lying free among the bacilli. The bacillus is motile and is provided with flagella. The tetanus bacillus usually maintains its existence at the depths of septic wounds and for this reason is difficult to identify and isolate. One method of isolation presumes the existence of spores which are very resistant to heat and on this supposition a series of melted agar tubes are inoculated at varying temperatures in the hope that other organisms will be destroyed and the tetanus spores survive. Fildes' method takes advantage of the habit of the bacillus to form a rapidly spreading film. In this method the local lesion is if possible excised, placed in a mixed beef medium and incubated for 3 days, the culture is then heated for 1½ hours at 65° C. to destroy aerobes and in particular *B. proteus* and a drop is transferred to the bottom of a well-dried sloped tube of influenza medium. After 24 hours' incubation a film growth will have spread up the medium from which the tetanus bacilli can be picked off in pure culture. All culture tubes must be grown under strictly anaerobic conditions. In agar and gelatin stab cultures the bacillus sends radiating spikes into the media and the gelatin is slowly

liquefied Such cultivation of a bacillus with terminal spores is extremely suggestive of the tetanus organism the positive proof however rests with animal inoculation Subcutaneous inoculation of the bacilli into a mouse produces tetanic spasms in 24 hours and death in 3 days The tetanus toxin is an extremely powerful one and like the diphtheria toxin is extracellular

B. botulinus resembles the tetanus bacillus in that it has little local action on the tissues but produces a powerful extracellular toxin with an affinity for the nervous system Further it is flagellated produces terminal or subterminal spores liquefies gelatin forms gas and is a strict anaerobe The bacillus is an important food poisoning agent and numerous cases in other countries have followed the ingestion of tinned meats olives or vegetables though epidemics in Great Britain are almost unknown The action of the toxin is upon the medulla and is apparent within a few hours of taking the infected food

B. Welchii (*B. aerogenes capsulatus*) is present in cultivated soils and in feces and is the most important organism in the production of gas gangrene of infected wounds It is a large stout pleomorphic bacillus with subterminal spores in culture media and a well marked capsule in the tissues The bacillus grows readily on the ordinary media under anaerobic conditions it clots milk and breaks up the clot by the formation of gas bubbles sugars are actively fermented with abundant gas production gelatin is liquefied

It is non motile and hæmolytic

B. œdematiens is an important cause of gas gangrene and produces a powerful toxin to which an antitoxin can be prepared Both toxin and antitoxin are distinct from those associated with *Vibrio septique* *B. œdematiens* produces a turbidity in glucose broth which clears after a day or two with the deposit of a flocculent mass at the bottom of the tube Gelatin is not liquefied

B. septique (*Vibrio septique* *B. œdematis maligni*) is a common cause of gas gangrene The bacilli are more slender than the foregoing and sometimes curved The spores are oval and subterminal Abundant gas is produced in culture gelatin is liquefied

B. chauvœi is the cause of black leg in cattle and less often in sheep It is not pathogenic to man

TABLE V *The Gram negative Bacilli*

	1		2.
<i>Hæmophilus</i>	Influenza	{ require X and V factors	Morax Axenfeld
	Koch Weeks		Ducrez
	Pertussis—V		Trachoma
		not required	{ Minute cols on blood agar
	3		
<i>Brucella</i>	Melitensis	{ slow growth on agar	
	Abortus		
	Tularensis—special media.		

4A Do not liquefy gelatin

		Motility	Inocul	Milk	Lactose	Dextrose	Mannit
<i>Bacterium</i>	Typhoid Fever	Typhoid	+	—	Acid	—	Acid
		Paratyphoid A	+	—	Sl acid	—	Acid gas
		Paratyphoid B	+	—	Acid to alkaline	—	Acid gas
	Food Poisoning	Gaertner	+	—	Acid to alkaline	—	Acid gas
		Aertryck	+	—	Acid to alkaline	—	Acid gas
	Dysentery	Shiga	—	—	Sl acid	—	Acid
		Flexner	—	±	Sl acid	—	Acid
		V W X Y Z	—	±	Sl acid	—	Acid
		Sonne	—	—	Acid clot	±	Acid
		Morgan	+	+	Alkaline	—	Acid gas
		Friedlander	—	—	Acid clot	Acid gas	Acid gas
		Acidi lactici	—	+	Acid clot	Acid gas	Acid gas
		Fæcalis alcaligenes	+	—	Alkaline	—	—
		Coli	+	+	Acid clot	Acid gas	Acid gas

4B Liquefy gelatin

Proteus—*Proteus*
Pseudomonas—*Pyocyaneus*

5

Vibrio—*cholera*
Pfeifferella—*Mallei*
Pasteurella—*Pestis*
Bartonella—*bacilliformis*

The pathogenic Gram negative bacilli are represented in a number of genera and differ widely from each other in their morphology and in their cultural and pathogenic actions. They are here divided into a number of different groups, in most of which the members have obvious relationships, but the classification of some Gram negative bacilli is still uncertain. Here and throughout this chapter the American classification and nomenclature are indicated though not strictly adhered to.

Group 1

This group consists of three members, all of which are minute bacilli, sometimes included with the members of the following group in the genus *hæmophilus*, a name which should

properly include only the first two members here described namely *B influenzae* and *B Koch Weeks*

B influenzae (*Pfeiffer's bacillus*) (Plate VII) In films made from the sputum the bacilli appear as tiny rods, often in clumps of considerable size, and many of them are found within the phagocytes. The organisms are present also in many of the complications of influenza, and may be obtained in pure culture from empyema pus and from joint fluids. Purulent spinal fluids containing influenza bacilli in large numbers are occasionally met with and are most frequently derived from young children. The associated condition is nearly always fatal. The organisms in these fluids are commonly pleomorphic and very long undulant forms are present. The influenza bacillus will not grow upon ordinary media but requires two growth substances, both of which are present in blood. Of these substances the "X factor" is heat stable and associated with hemoglobin the "V factor" is heat labile and present in red cells and in yeast. Nearly all known bacteria are capable of elaborating the V factor during growth, the influenza and the Koch Weeks bacilli require the V factor to be supplied to them. The influenza bacillus will therefore grow on agar to which fresh blood has been added or on a hematin containing medium in symbiosis with other bacteria to supply the V factor. Bacilli of similar appearance, which, unlike the influenza bacilli are haemolytic and require the V but not the X factor are sometimes met with and are probably non pathogenic. A simple medium for the growth of the influenza bacillus is an agar slope streaked with fresh blood on which the colonies are very small and very translucent, being only just visible to the naked eye. A better medium is that of Fildes (p. 251) and the isolation of the organism from a mixed source, such as the sputum is best attempted on plate cultures of this medium. The relationship of the bacillus to epidemic influenza is still in dispute. The transmission of a febrile state to human volunteers by means of bacteria free filtrates of mouth and nose washings from influenza cases and the transference to ferrets (the only animal so far discovered to be susceptible) of a fever of constant type with similar material points to a virus as the causative agent of the epidemic and the bacillus as a secondary invader. This conception is in close analogy with swine influenza, the virus of which produces a mild disease, but when associated with an influenza organism a serious and often fatal one. In

the human epidemic it is probable that the influenza bacillus is not the only secondary invader since streptococcal pneumococcal and staphylococcal complications are frequently met with

Koch Weeks bacillus This bacillus is by far the most common cause of acute contagious conjunctivitis. The organisms are identical in appearance with the influenza bacillus and are found in large numbers in films made from the conjunctival discharge. The cultural properties of the bacillus are those of Pfeiffer's organism and there are no bacteriological methods of distinguishing between the two bacteria.

B pertussis A great variety of organisms have been described as the essential cause of pertussis. The bacillus referred to here is that isolated by Bordet and Gengou and considered by them to be the causative organism. The bacillus which is found in considerable numbers in the sputum in the early stages of whooping cough is a minute one closely resembling the influenza bacillus but in culture has less tendency to produce involution forms and on sub culture grows more abundantly. The organism grows well on blood agar but the presence of hæmoglobin is not essential and in sub culture growth takes place in the absence of both λ and V factors. The bacillus is agglutinated by the sera of convalescent cases and complement fixation tests are positive. Isolation of the organism in plate cultures of the sputum in the diagnosis of the disease and the use of vaccines both in prophylaxis and treatment are procedures to which more value has been attached on the Continent than in England.

Group 2

In this group are three unclassified bacteria all of which have some cultural or morphological resemblance to the members of the preceding group.

The Morax Axenfeld bacillus is the cause of diplo bacillary or angular conjunctivitis and may be recognised in the conjunctival discharge as a fairly large stout bacillus with rounded ends mainly in pairs but also in short chains. It grows in small translucent colonies on serum or ascitic agar.

Ducrez's bacillus is the cause of the soft chancre and in the discharge is found as a minute bacillus in pairs and short chains.

It is grown with difficulty from the tissues but can be cultivated on rabbit blood agar

Trachoma A minute bacillus was cultivated by Noguchi from cases of trachoma and considered by him to be the cause of the disease. The organism can be grown on blood agar and cultures inoculated into monkeys have produced trachoma like lesions. The relationship of the bacillus to the disease is still *sub judice*.

Group 3

The third group of Gram negative bacilli is represented by three bacteria of very similar morphology. They are minute non motile organisms presenting coccoid and bacillary forms. All produce diseases in animals and are only incidentally conveyed from them to man.

Br melitensis This bacillus was shown by Bruce to be the causative organism of Malta fever and to be conveyed to man by the drinking of goat's milk. The disease in man once very prevalent in the Mediterranean has been almost eliminated. It is a long-continued remittent fever accompanied by joint pains and splenic enlargement. The organism grows upon the ordinary media but slowly and on agar slopes the small translucent colonies take some 2 or 3 days to develop. Growth is more abundant on glucose agar and is best maintained on this medium. Milk is not acidified and there is no liquefaction of gelatin. Cultures of the organism are notoriously infective to laboratory workers. The bacillus is readily grown from the milk of the carrier goats from blood cultures in human cases and is intermittently present in the urine. Agglutinins appear in the blood towards the end of the first week of the fever and commonly reach a very high titre.

Br abortus produces epidemic abortion in cattle and is excreted in the milk. It has been proved to be the causative agent in numerous cases of undulant fever in man in all countries and in particular in rural districts where non pasteurised milk is drunk. Human beings are more resistant to infection by this organism than to *Br melitensis*. *Br abortus* cannot be distinguished on morphological grounds from *Br melitensis* and its cultural behaviour is very similar. It is nearly always essential however to incubate the primary culture of *Br abortus* in a partial atmosphere of CO_2 (an increase of 10 per cent by volume) but after several sub

cultures the organism will grow in atmospheric air. The addition of gentian violet to the medium in a concentration of 1 in 50 000 will usually inhibit *abortus* and not *melitensis*. In cases of infection the organism can be grown from the blood often up to a late period and agglutinins reach a high titre. In brucella infections occasional cases are met with in which agglutinins are absent and some strains of the organisms agglutinate with normal sera. The agglutination reaction is however of the greatest value in the common diagnosis of fevers due to all members of this group but of little help in differentiating between them since cross agglutination is the rule and in the cases of *Br melitensis* and *Br abortus* absorption tests indicate a variety of strains of both organisms rather than a separation of two specific types. The somewhat inconstant differences between *melitensis* and *abortus* suggest that the two bacteria are modified from a common stock by passage through different animals.

Br tularensis (*Bacterium tularense*) was first recognised as the cause of epidemic disease among ground squirrels and jack rabbits in the United States. It has been discovered since in other rodents and in game birds and in many parts of the world. The infection is conveyed to man by inoculation as in preparing a rabbit for cooking or by insect bites. The condition in man is known as tularaemia and runs a course like that of *abortus* infections but with the frequent addition of a local septic condition at the site of inoculation. *Br tularensis* is identical in appearance with the other members of this group but will not grow on simple media. It can be cultivated on an egg yolk medium or on rabbit blood glucose agar to which 0.1 per cent of cystine has been added. The organism is not easily isolated from human cases and diagnosis usually rests upon the high agglutination titre of the sera. The classification of *Br tularensis* is not yet determined but its morphological and serological affinity to the brucella group is close.

Group 4A

The fourth group of Gram negative bacilli comprises a considerable variety of organisms all of which grow readily in simple media in rod shaped forms of like appearance. The group can be divided on the basis of one important cultural characteristic namely the manner of growth on gelatin. The bacilli of the first division include all the important organisms

of the coli typhoid group and do not liquefy gelatin. The bacilli of the second division are of less pathological significance, and all liquefy gelatin. Group 4A contains the typhoid and paratyphoid bacilli, the food poisoning bacilli, the dysentery bacilli, and the colon bacilli. They are classified in the genus *Bacterium*.

B typhosus (*Bacterium typhosum*) The typhoid bacillus is an actively motile organism provided with numerous flagella. Rapid growth is readily obtained on all the usual cultural media. The more important cultural characters are the following. On agar slopes a growth is produced which is rather more translucent and has less tendency to lateral spread than in the case of the colon bacillus. In broth a general turbidity is produced but no indole. In litmus milk a faint acid reaction is set up but no clotting takes place. No gas is produced in any of the litmus carbohydrate media but litmus dextrose is acidified. Neutral red broth is unchanged. Yellow colonies are produced on MacConkey's neutral red bile salt lactose agar medium and the surrounding medium subsequently turns yellow. Identification of the bacillus rests in the first place upon its motility and cultural characters but finally upon its agglutination reactions. The serological behaviour of the typhoid bacillus is very constant and it is rarely necessary to carry out absorption tests. A motile bacillus recovered from the body which has the proper cultural characters and is agglutinated to titre by an antityphoid serum may be regarded as the typhoid bacillus and in cases of doubt the bacilli can be shown to remove the agglutinins from the serum after absorption. Occasionally a typhoid bacillus freshly isolated is not agglutinated but usually becomes agglutinable on subcultivation. (See also p 175.)

Typhoid fever is spread either by direct contact such as may occur in nursing a patient or more commonly through the intermediary of a carrier or by an infected water supply. The disease is in the first instance a general blood infection and the bacilli may be readily isolated from the blood in the first week of the disease and during a relapse. Later the bacilli are found in the faeces and less commonly in the urine. The bacilluria which may complicate the late stages of typhoid fever is usually set up by the colon bacillus and far less commonly by the typhoid bacillus. The bacilli may be obtained from the gall bladder many years after the attack.

and are present in the bone abscesses which may follow the attack. The bacilli may persist in the faeces, and less commonly in the urine many years after the primary infection and without injury to the host. Persons thus infected are known as 'typhoid carriers' and are a source of grave danger to the community.

The laboratory diagnosis of typhoid fever can be made in the first week of the disease by means of a blood culture. A few cubic centimetres of blood are taken into broth or into ox bile incubated for 12 hours and plated out on MacConkey's medium. The yellow colonies are then picked off and tested culturally and by the serum reaction. At the beginning of the second week the Grunbaum-Widal test becomes positive in the patient's serum and the bacilli may be isolated from the faeces.

Prophylactic inoculation against typhoid fever with dead cultures of the bacilli is advisably given to those compelled to live in countries where the disease is rife. The vaccine employed is preferably a mixed one containing typhoid with the paratyphoid A and B bacilli (p. 206). Persons thus inoculated should not abate the customary precautions taken with regard to their water supply since they are not necessarily immune against a heavy infection.

B paratyphosus The paratyphoid bacilli form a group of closely allied organisms which can be recognised generally by their cultural characters specifically only by agglutination tests. The name *Salmonella* is commonly given to the group. Four members are mentioned here — *B. paratyphosus* A, *B. paratyphosus* B, *B. aertryck*, *B. enteritidis* (Gaertner). The first two bacilli produce a disease which cannot be distinguished on clinical grounds from typhoid fever. Paratyphoid infections, however, commonly run a milder course than typhoid, are perhaps more contagious, are to be suspected when patients apparently suffering from typhoid fail to give positive Widal reactions, and are definitely diagnosed on the agglutination tests with the appropriate bacilli and on the isolation of the organisms from the blood or faeces. The paratyphoid B bacillus is the organism most commonly met with in this country and the A bacillus in India but after the war more numerous infections by paratyphoid A occurred in England. *B. aertryck* (*B. suispestifer*) and *B. enteritidis* (Gaertner) are commonly associated with meat poisoning epidemics, and produce

a disease with an incubation period of a few hours followed by acute gastro intestinal symptoms of comparatively short duration

The organisms as a group have the following cultural characters Acid and gas are produced from dextrose and mannite Lactose is unchanged No indole is formed Litmus milk becomes acid in the first 24 hours and then strongly alkaline Neutral red broth is turned yellow *B paratyphosus* A differs from the other members of the group in its behaviour in litmus milk in which it produces a permanent acidity but no clot

The bacilli can be differentiated one from the other by agglutination reactions but in the case of *B aertryck* and *B paratyphosus* B only by carefully performed absorption tests with standard sera on the lines indicated in a subsequent chapter (p 170) Suspected cases of typhoid fever which give in their sera negative or partial agglutination reactions with *B typhosus* should be tested upon known cultures of *B paratyphosus* B and A The three infections are sufficiently common and similar to warrant the testing of all suspected febrile cases against each of the three bacilli as a routine procedure The paratyphoid bacilli are found in the blood and in the faeces in the same manner as are the typhoid bacilli Cases of acute food poisoning should in the later stages of infection be tested for agglutinins against Gaertner's bacillus and *B aertryck* and the organisms sought for in the blood or faeces in the acute stage as well as in the suspected food

B dysenteriae The dysentery bacilli form a group of closely allied and widely spread organisms of which the main varieties are the *B dysenteriae* of Shiga and the *B dysenteriae* of Flexner These organisms are the causes of bacillary dysentery such as is met with in tropical and in temperate climates They are also associated with some forms of ulcerative colitis in this country and have been found in out breaks of asylum and other varieties of dysentery as well as in epidemics of infantile enteritis

The bacilli are non motile and do not appear to be provided with flagella Their chief cultural characters are as follows No gas is produced in any of the carbohydrate media Litmus glucose broth is acidified and lactose is unchanged Litmus mannite broth is acidified by the Flexner organism but not by the Shiga bacillus Indole is formed by the Flexner type only

Milk is rendered first acid and then alkaline. Neutral red broth is unchanged. The Shiga bacillus in cultures produces a soluble toxin which causes paralysis and death in rabbits. Filtrates of *B. Flexner* cultures are not toxic.

The Flexner group of dysentery bacilli can be further divided serologically into 5 groups labelled v w x y z which are agglutinated to titre by their appropriate sera but give rise to considerable cross agglutination between the groups.

B. Morgan No. 1 is an actively motile bacillus which forms indole in peptone broth and ferments dextrose with slight gas production. Morgan's bacillus appears to be associated with some dysenteric outbreaks and with infantile diarrhoea.

Sonne's bacillus is non motile, acidifies glucose, mannite and sometimes lactose, acidifies and clots milk and produces no indole. It appears to be a not infrequent cause of dysentery.

In suspected cases of dysentery the bacilli should be sought for in the stools and the patient's serum should be tested by ordinary agglutination methods upon the bacilli isolated and upon known strains of dysentery bacilli. In a positive serum reaction the bacilli are agglutinated somewhat slowly in dilutions of the serum up to 1 in 50 but the reaction is rarely complete before the twelfth day of the disease.

B. coli communis (Plate VIII). The colon bacillus like other members of this group has certain typical characters but occasional varieties are met with presenting differences of minor importance. The typical bacillus only will be considered here.

The colon bacillus has few flagella and is only sluggishly motile though active strains are occasionally met with. The organism can be identified by its cultural characters never from its morphological appearances alone. The main cultural reactions are as follows. Acid and gas are produced in the great majority of the carbohydrate media including dextrose, lactose and mannite. Indole is formed in broth. Milk is acidified and clotted. On agar slopes a thick greyish white streak is formed with spreading edges. On agar plates large circular colonies appear with heaped up centres and crenated margins. (The appearances on solid media are almost identical for all members of the coli typhoid group except that the typhoid growth is slightly more delicate while the other organisms occupy an intermediate position.) The colour of neutral red broth is changed to a canary yellow, and a green

fluorescence is produced in the medium. Red colonies are formed on MacConkey's bile salt medium.

The colon bacillus is a normal inhabitant of the large intestine but in abnormal situations it produces suppuration and disease. The more important affections associated with this organism are those connected with intestinal lesions as for example the general peritonitis which follows a perforated appendix. The colon bacillus frequently gains entry into the urinary tract particularly of females where it may be latent producing no symptoms or may give rise to severe suppurative nephritis pyelitis or cystitis. The bacillus is also found in diseased processes in the gall bladder (often in association with calculi) in the bile passages and in numerous other parts of the body. It has been isolated in pure culture from the cerebro spinal canal. From any situation in which colon bacilli are producing infection the organisms may enter the blood in sufficient numbers to be easily detected in cultures. Such entry is usually accompanied by a rigor and after a few hours the blood is again sterile.

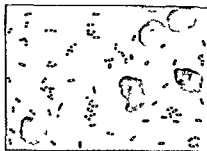
The pus in which the colon bacillus is found is frequently most offensive owing to the fact that since these lesions are so often in communication with the gastro intestinal tract there are present in addition to the colon bacilli certain long thin delicate saprophytic bacilli which normally inhabit this tract and are capable of producing the most virulent odour.

Serum reactions with the bacillus are unsatisfactory since an appreciable increase in agglutinin is not commonly present and the sera of infected persons rarely agglutinate the bacillus in dilutions of more than 1 in 10.

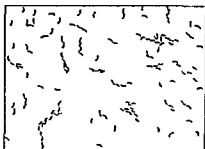
In the examination of urine infected wounds and particularly of faeces bacilli of atypical cultural characters are frequently encountered. While many of these atypical bacilli are in reality mixed strains due to imperfect methods of isolation the number of bacilli of the coli typhoid group to which specific names have been given is very large and mention can be made of a few only.

B. coli anaerogenes resembles the typhoid bacillus more closely than the colon bacillus in that it does not produce gas. It differs from the typhoid bacillus by its agglutination reaction and in acidifying lactose.

B. acidilactici closely resembles *B. coli* in its cultural characters and is one of a group of bacteria concerned in the souring of milk. Another named organism of the same class



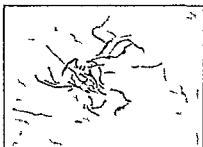
B. Pestis
In ear from Spleen (Arbitration)



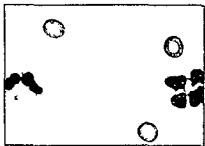
Cholera Vibrio
Lact Culture (Carbol thion)



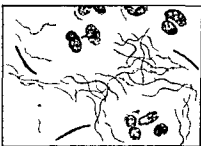
B. Coli.
Lact Culture (Arbitration)



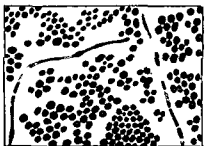
Actinomyces.
Lact Culture (Carbol thion)



Spirochaeta Pallida *
Lact Culture (Carbol thion)



Spirilla and Fusiform Bacilli (Vincent)
From Septic Mouth (Carbol thion)



Oidium Albicans
From Agar Culture (Carbol thion)



Sarcinae
From Agar Culture (Carbol thion)

* The shape and regularity of the spirals are to a large extent lost in the process of reproduction

is the *bacillus lactis aerogenes* which differs from the first named in fermenting saccharose

B Friedlander (*Friedlander's pneumo bacillus*) has been isolated from various lesions and is a rare agent in the production of pneumonia. In its general cultural characters the organism closely resembles *B coli* but differs from it in being capsulated and in its sticky mucoid growth on solid media. The capsule contains a polysaccharide which differs in different types of the organism as is the case with the types of pneumococci. One type of *B Friedlander* is immunologically similar to Type II pneumococcus.

B *faecalis alcaligenes* is a frequent inhabitant of the intestinal tract and is a rare cause of infection in other parts of the body. Its cultural characters closely approach those of the typhoid bacillus but it produces a characteristic and strong alkaline reaction in litmus milk and can readily be distinguished by serological means.

Lastly organisms closely resembling the paratyphoid group in their cultural characters but readily differentiated by agglutination tests are frequently met with in the routine bacteriological examination of urine and faeces.

NOTE The student is scarcely expected to remember in detail the various cultural characters of the organisms in this group (Group 4A). He should however remember the main distinctions between the typhoid and colon bacilli. A reference to the bacteriological table affords a simple guide to these characters. The bacilli are placed from above downwards very approximately in order of their virulence, their specificity and their properties of producing agglutinins. The cultural characters proceed in the opposite direction, thus the colon bacillus alters almost every medium in the greatest possible manner, the typhoid bacillus has the least effect upon the media and the other organisms occupy an approximately intermediate position.

Group 4B

This group contains two bacilli which usually lead a saprophytic existence but may on occasion either alone or more commonly in association with other bacteria produce disease.

B proteus (*B proteus vulgaris*) This name is given to a number of closely allied organisms identical in appearance with the colon bacillus. They are actively motile and on solid media

tend to spread in a thin film over the surface. They produce acid and gas in dextrose but do not change lactose and mannite. Litmus milk is clotted by some strains and not by others. the litmus is reduced. Indole formation is not constant. *B. proteus* is commonly found in the faeces and in the urine. In septic infections of the urinary tract the organism may be recovered in pure culture or more commonly in association with the colon bacillus. The presence of *B. proteus* in a carefully taken catheter specimen of urine is suggestive of some underlying organic lesion such as tuberculosis malignant disease or calculus since a primary infection with this organism unlike a primary infection by the colon bacillus is most unusual.

The relationship of proteus X 19 to typhus and the rickettsiae is discussed elsewhere (p. 142).

B. pyocyaneus. This is an actively motile bacillus with a tendency to spontaneous agglutination in hanging-drop preparations. The organism is readily recognised in cultures by the blue green pigment which it produces and which diffuses out into the medium. The pigment consists of two substances one of which is soluble in chloroform and bright blue in colour. Acid and gas are produced in the majority of the carbohydrate media.

B. pyocyaneus most frequently occurs as a secondary invader often in association with lesions connected with the intestinal tract as in ischio rectal abscess or in general peritonitis secondary to a gangrenous appendix. The bacillus may also be associated with cerebral abscess a spreading cellulitis or a local abscess. The pus in these infections is of a blue colour. The lesions associated with *B. pyocyaneus* are often somewhat intractable and a general peritonitis accompanied by this bacillus is almost invariably fatal. On the other hand intractable wounds may for the first time begin to heal after a secondary infection by *B. pyocyaneus* has taken place. After inoculation into the peritoneal cavity of a guinea pig a virulent and rapidly fatal peritonitis follows.

Group 3

Under this heading is considered a variety of bacteria many of great pathogenic importance which bear little or no relationship to each other but which cannot reasonably be attached to any of the foregoing groups.

V. cholerae (*comma bacillus*) (Plate VIII) The vibrios are short, curved, flagellated and motile organisms, most of which are non pathogenic. The comma shape in freshly isolated cultures is striking and the organisms tend to lie with their long axes in the same direction. In older cultures stunted and bacillary forms occur. The cholera vibrio grows readily on all the ordinary media, and on gelatin plate cultures, before liquefaction has become advanced, form irregular granular colonies like "fragments of broken glass". In broth a pellicle is produced on the surface of the medium and both indole and nitrites are formed from the peptone, so that a pink colour (the cholera red reaction) appears on addition of a few drops of pure sulphuric acid. Litmus milk is unchanged. The differentiation of the organism from other vibrios must be completed by the agglutination reaction or Pfeiffer's test (p. 388).

In the human body the bacteria are confined to the intestine, and in the acute cases the watery stools appear to form an almost pure culture of the organism. Numerous other varieties of spirilla have been described, and are of particular interest in that they may be mistaken for the cholera vibrio. Metchnikoff has recorded a similar vibrio found in the intestines of fowls dead of gastro enteritis. Finkler and Prior described another present in infantile diarrhoea. Vibrios distinct from the cholera spirillum have been found also in the mouth, and in the water supply of numerous towns. It is evident, therefore, that considerable caution should be exercised in the identification of the genuine vibrio, particularly in the absence of an epidemic.

B. mallei (*Pfeifferella mallei*) The bacillus of glanders mainly affects horses, and very exceptionally produces disease in man. Infection in man starts from a local abrasion on the skin or the nasal mucous membrane and spreads by the lymphatics, giving rise to an acute or chronic pyæmic condition, with secondary abscesses in the tissues, lungs, or joints. The bacilli are slender, curved rods, staining faintly, and often in a beaded manner, with the ordinary dyes. In film preparations they are mainly extracellular. The organism grows on the ordinary media, but somewhat slowly. On agar and blood serum growth appears as a shiny, greyish streak in two days. On potato a membranous growth is formed, which by the eighth day becomes a reddish brown colour. Inoculation into the abdominal cavity of a male guinea pig is followed by

peritonitis swelling of the testicles, and a purulent exudate into the tunica vaginalis. The appearance and staining reactions of the organism, the growth on potato and the effect of inoculation into a guinea pig should all be investigated before making a diagnosis.

B. pestis (*Pasteurella pestis*) (Plate VIII) The plague bacillus is one of a small group of bacteria, of which *pestis* produces plague in man, *aviseptica* hæmorrhagic septicæmia in fowls and *pseudotuberculosis* a disease of rodents. All are closely connected, and although distinction can usually be made on serological grounds with low titre sera, there is considerable cross agglutination. The chief cultural distinctions between *pestis* and *pseudotuberculosis* are the production of alkalinity in litmus milk, motility in broth culture and the lack of pathogenicity for white rats with the latter organism. *Pestis* is non motile, produces no change in milk and is highly pathogenic to white rats. The bacillus of plague is mainly spread to man from the rat by the intermediary of the rat flea. The disease in man is of three main types—the bubonic, in which the lymphatic glands are affected, the pulmonary which almost invariably terminates in a fatal septicæmia and the septicæmic. The bacilli are found in large numbers in films made from gland pus or sputum, and stain deeply at each end and faintly in the centre, each bacillus having the appearance of a diplococcus. This polar staining is best seen if the films are first fixed in absolute alcohol. *B. pestis* grows on the ordinary media forming a poor, greyish yellow growth on agar. Gelatin is not liquefied. Broth shows a granular deposit and the bacillus grows in chains. The disease can be reproduced in mice and guinea pigs by inoculation.

Bartonella bacilliformis is a small bacillus found within the red cells in cases of the Oroya fever of Peru, and is also the cause of the chronic skin condition *verruca peruviana*. It is a motile organism and can be cultivated on serum hæmoglobin agar medium. *B. muris-ratti*, a similar intra corpuscular bacillus occurs in rats and may not affect the rats unless the spleen is removed when the bacilli invade the red cells and produce anæmia. *B. canis* is the cause of an infectious anæmia of dogs.

Bacillus prodigiosus (*Chromobacterium prodigiosum*) is one of a considerable group of pigment producing bacteria. It is non pathogenic but is of importance because of its wide distribution in water and air, and in consequence its appearance

as a contaminant in cultures. Also, because of its small size, the polished bright red colour of its colonies on agar and its harmless character, *prodigiosus* is commonly used as a test organism for the permeability of bacterial filters and for leakages in water systems.

CHAPTER VII

SPIROCHÆTES—FILTERABLE VIRUSES— RICKETTSIÆ

THE SPIROCHÆTES

THE spirochætes form a considerable group of organisms which are generally considered to be more closely related to the bacteria than to the protozoa. They are spiral flexible and motile but not flagellated organisms and they multiply by transverse division. The majority are difficult to culture and some are so thin as almost to pass the limits of microscopic vision and to traverse bacterial filters. The spirochætes of importance in human pathology are conveniently divided into three main groups—the saprophytic, the blood and the tissue spirochætes.

TABLE VI

Saprophytic	Blood Spirochætes	Tissue Spirochætes
<i>S. buccalis</i>	<i>S. recurrentis</i> (relapsing fever)	<i>S. pallida</i> (syphilis)
<i>S. vincenti</i>		<i>S. pertenuis</i> (yaws)
<i>S. dentium</i>	<i>S. duttoni</i> (African tick fever)	<i>S. icterohæmorrhagiae</i> (Infectious jaundice)
<i>S. refrigens</i>		<i>S. minus</i> (rat bite fever)

Saprophytic spirochætes Many spirochætes under many different names have been described in the mouth, the lungs, the intestinal tract and in the genitalia. All so far as they can be identified maintain an existence in the absence of disease and tend to multiply greatly in the presence of inflammation. The influence of the spirochætes upon the tissues and the associated bacteria in septic conditions is not known.

S. buccalis is a large and somewhat coarse spirochæte with irregular spirals found in the mouth and in the sputum. It may be present in large numbers in the pus from bronchiectatic cavities and is often associated with a fusiform bacillus.

Bronchial spirochætosis has been widely written of as a distinct infection but the evidence is far from conclusive

S. vincenti (Plate VIII) is an organism similar in appearance to and possibly identical with *S. buccalis*. It has been cultivated on serum agar under anaerobic conditions. It is commonly associated with the fusiform bacillus of Vincent, a coarse Gram negative bacillus with thickened centre. The bacillus is a strict anaerobe and more easily grown in symbiosis. The association of the spirochætes and the bacilli is probably symbiotic and not, as has been claimed, a difference of developmental stage in the same organism. Vincent's organisms stain with simple dyes and can be found in any buccal cavity if looked for in direct smears. In septic conditions they may increase enormously and in some forms of ulcerative stomatitis or tonsillitis particularly in children may form a thick network in the film made from a swab. The clinical condition is known as Vincent's angina but since streptococci and other pyogenic bacteria can nearly always be recovered from these ulcerative and gangrenous lesions and since Vincent's organisms are so universally distributed it is doubtful if the spirochætes and fusiform bacilli are more than secondary invaders.

S. dentium is a very delicate spirochæte often with 8 or more fairly regular spirals. It is commonly found in the mouth.

S. refringens is a spirochæte of the genitalia and chiefly of importance because it may be confused with *S. pallida*. *S. refringens* is larger, thicker and more actively motile than *S. pallida*. In films it stains with the ordinary dyes and shows great irregularity of spirals. Minute spirochætes of the *S. dentium* type are also though more rarely found on the genitalia.

Blood spirochætes. Spirochætal conditions in which the organisms can be readily demonstrated in blood films are found in all classes of animals and in man relapsing fevers due to spirochætes occur in all parts of the globe. The spirochætes of all types of relapsing fever are morphologically indistinguishable. They vary considerably in size with an average length of 15μ and an average number of 5 to 6 spirals. The extremities are slightly pointed and the organisms are highly motile and can penetrate tissue cells. They are found in the cerebrospinal fluid as well as in the blood. In blood films the spirochætes can be readily demonstrated in ordinary Leishman

preparations but tend to stain faintly. During the febrile period they are very numerous and during the remissions extremely scanty. They are aerobic and can be cultivated on Noguchi's medium (p. 252). The usual method of infection is by lice or ticks.

S. recurrentis (*S. obermeieri*) is the cause of European relapsing fever, which is still common in Russia and other parts of Europe. The disease is accompanied by febrile attacks of 5 to 7 days' duration, followed by periods of apyrexia and one or two relapses. The spleen is usually enlarged. The mortality rate is low, rarely exceeding 5 per cent and the infection is readily amenable to salvarsan treatment, though arsenic resistant strains of the spirochæte may be produced.

S. duttoni is the cause of the tick transmitted fever of tropical Africa. The tick concerned is *Ornithodoros moubata* and the infection in the ticks is hereditary. The fever in man is generally more severe and the relapses more numerous than in European relapsing fever. Similar relapsing fevers spread by lice or ticks and caused by morphologically identical spirochætes have been given names corresponding to the localities in which they occur, but in all cases the causative organisms are either identical or so closely related that no differentiation is possible.

Tissue spirochætes. The tissue spirochætes are not confined to the tissues nor are the blood spirochætes to the blood, but the organisms of this group produce their main effects in the tissues and can be demonstrated there, appearing in the blood in such small numbers that they can rarely be found in blood films. The group includes the organisms of syphilis, yaws, infectious jaundice and rat bite fever. According to the classification of Noguchi the spirochætes of syphilis and yaws belong to the genus *Treponema*, are provided with axial filaments and are soluble in saponin and distilled water, as are also the blood spirochætes. The spirochætes of infectious jaundice have no axial filaments which can be distinguished by staining are resistant to saponin and distilled water and are classed in the genus *Leptospira*. The organism of rat bite fever has a comparatively rigid body and terminal flagella, it is at present included in the genus *Spirillum*. The classification of the spirochætes is however a changing one, and it is common and convenient to refer to all of these and the other groups as spirochætes.

S pallida (*Treponema pallidum*) (Plate VIII) *S pallida* is the causative organism of syphilis and its identification particularly in the primary chancre is of the first importance since the clinical diagnosis of primary syphilis is never certain until it has been confirmed bacteriologically. In the fresh exudate examined by dark ground illumination (p 230) the spirochæte of syphilis appears as a slowly moving extremely delicate organism with numerous regular spirals which do not straighten out during rest. In Indian ink preparations (p 229) the spirochæte has a very similar appearance but has of course no movement. It can be demonstrated in dried films by one of the modifications of Fontana's method (p 230) which blackens and thickens the spirochæte. The spirochæte is left unstained by the ordinary methods but is well stained by Giemsa's dye after preliminary fixation with absolute alcohol (p 229). The appearance in Giemsa preparations is very characteristic. The *S pallida* stains pink in contradistinction to the majority of other spirochætes which stain purple. The minute delicate spirals are numerous—from 6 to 20—and very regular. By this method the specific morphology of the syphilitic organism is sharply contrasted with that of the saprophytic spirochætes but the method has the disadvantage that many of the spirochætes become lost in the preliminary alcoholic fixation and the organism is so delicate that experience and care are needed to detect it. Of all the methods in common use the dark ground is most generally convenient provided the examination is often enough called for to justify the permanent assemblage of the apparatus. For occasional examinations the Giemsa stain is more laborious but possibly more diagnostic than the Fontana method. The spirochætes are usually present in considerable numbers in both primary and secondary lesions. They are very scanty in the lesions of tertiary syphilis. The *S pallida* cannot be cultivated on the usual media but in common with the majority of other spirochætes can be made to grow in media specially devised for the purpose. Owing to the presence of contaminating bacteria the isolation of *S pallida* in pure culture is rarely successful even in skilled hands. Inoculation of fluid containing the organisms into one of the higher apes is followed by a local chancre and later by secondary manifestations. Inoculation into the lower monkeys or into the testicle of a rabbit is followed by a local sore only which tends to heal spontaneously.

The early diagnosis of syphilis by the demonstration of the spirochæte in the primary chancre *before the serum reaction has become positive* is of the utmost importance since the cure of the disease depends so much upon the promptitude with which treatment is commenced. The success of the demonstration rests largely upon the care with which the material to be examined is obtained. The surface of the chancre should first be washed over thoroughly with sterile salt solution and an attempt made by vigorous squeezing of the edges of the ulcer to cause a clear or blood tinged serum to exude from the depths of the base. If the chancre is very painful or firmly encrusted over the surface should be well swabbed with 4 per cent eucaine and a further attempt made to obtain the proper fluid aided if necessary by a scraping of the chancre base with the edge of a glass microscope slide. The fluid is transferred in a platinum loop to a slide and both stained and fresh specimens should be prepared. The greatest care should be taken to avoid infection and the examiner should never touch a suspected chancre with bare fingers. Any handling of the infected part can safely be done by the patient.

A negative examination is not sufficient contra indication of syphilis but in skilled hands negative results in syphilitic cases are unusual. In cases of doubt a second examination should be made and if enlarged glands are present in the groin the most prominent should be punctured with a hypodermic needle and the minute quantity of fluid thus obtained often yields a positive result. The recognition of the *S. pallida* is positive evidence of syphilis provided the identification of the organism is correct. Numerous other varieties of spiral organisms are to be found in the body particularly in the region of the mouth the anus and much less frequently the male urethra and the vulva. Particular care therefore should be exercised in the examination of the majority of extragenital chancres. The main points in the identity of the syphilitic organism are the comparative lack of motility the failure to stain by the ordinary dyes and the rose pink colour when stained by Giemsa's dye the delicacy of the spirochæte the large number of its spirals (from 12 upwards should be counted) and their regularity.

S. pertenuis (*Treponema pertenuis*) is morphologically indistinguishable from *S. pallida* and from *S. cuniculi* a common spirochæte specific for rabbits and producing in them a local ed

inflammation of the skin *S. pertenuis* is the causative organism of yaws, a tropical disease spread by direct contact in which the primary lesion is extragenital and the secondary lesions mainly limited to the skin. It is commoner in rural than in urban districts and rare in Europeans. A positive Wassermann reaction is the rule and the relationship of yaws to syphilis has not been definitely settled. The spirochæte is easily found in recent lesions.

S. icterohæmorrhagæ (*Leptospira icterohæmorrhagæ*) is the cause of Weil's disease or infectious jaundice. It is a slender spirochæte with a length of from 7 to 14 μ and an average of two spirals to the micron. It can be cultivated in rabbit serum agar media kept at 28° C. The spirochætes are very difficult to find in blood films but can be obtained in culture from the blood during the first few days of the fever. In the second or third week the organisms appear in the urine. Diagnosis can be confirmed by inoculating a guinea pig with blood or the urinary deposit. After subcutaneous inoculation the pig becomes jaundiced and rarely survives more than 10 days. Spirochætes are usually abundant in the blood and urine.

Spirillum minus (*Spirochæta morsus muris*) is the causative organism of rat bite fever or of Sodoku in Japan. The organism can be found in the local sore or by puncture of a neighbouring lymph gland; it is extremely scanty in the peripheral blood. The cultivation of *S. minus* has been rarely successful. Rat bite fever is usually accompanied by a positive Wassermann reaction and is readily cured by salvarsan.

FILTERABLE VIRUSES

The investigation of problems concerned with the filterable viruses is the concern of the specialist and takes no place, as yet, in routine clinical pathology. These agents of disease are of such great interest and importance, however, that the following general account of them is given to enable the student to gain some conception of this important branch of microbiology.

In many diseases, not only of man but of other animals and plants, it has been impossible to demonstrate a causal agent belonging to the cultivable bacteria. These diseases have all the features of an infective process, many of them are highly

contagious, and they constitute a group of the greatest importance both medically and economically. In 1891 Ivanowsky found that the agent of mosaic disease, a wide spread disease of plants characterised by a mosaic like mottling of the leaves, was capable of passing through filters which retained the ordinary bacteria. This observation was confirmed by Beijerinck, and in 1898 Löffler and Frosch published their classical work on foot and mouth disease, showing that the same held true for the causal agent of this condition. These investigations gave birth to the conception of filterable viruses and since that time the number of examples has been multiplied to the extent that to-day more than a hundred diseases of animals and plants are attributed to agents of this kind. It must be recognised that although the term 'filterable virus' is established by usage, it is by no means an ideal one. Even were filterability solely a function of size of particle it would still be unsatisfactory for we know now that from the smallest filterable virus up to the largest cultivable bacterium there is an unbroken gradation in size, the one group merges almost imperceptibly into the other. The alternative term of ultra microscopic virus is also unsatisfactory, for some of the larger viruses come within the limits of resolution with the microscope. It would probably be best to follow the modern tendency to drop both of these adjectives and speak of this group of disease agents simply as 'viruses'. This vague term is no longer necessary when referring to the pathogenic bacteria and protozoa so that its use in this restricted sense is not likely to cause any confusion.

Amongst the virus diseases of man are measles, mumps, smallpox, chickenpox, zoster, herpes, *molluscum contagiosum*, warts, dengue, yellow fever, the common cold, *poliomyelitis* and probably also influenza and *encephalitis lethargica*. Examples of virus diseases of other animals are rabies, distemper, the animal pox diseases, of which cowpox is of greatest interest to medicine, foot and mouth disease, swine fever, louping ill of sheep, fowl leukæmia and psittacosis, and in plants we have mosaic disease, streak, leaf curl, yellows and other yet ill-defined conditions. Some of the animal virus diseases are communicable also to man. Cases of human infection with the viruses of rabies and psittacosis are well known, and the ease with which the latter disease passes from parrot to man was well exemplified in the recent world epidemic.

Foot and mouth disease in man, though rare, does undoubtedly occur

Characters of the virus group. (1) *Size* Although it has been said that, on the score of size, no clear dividing line can be drawn between the viruses and the cultivable bacteria, it is correct to say that taken as a group the viruses are much smaller than the cultivable bacteria. Accurate ultra-filtration studies have established that the viruses range in size from $10\text{m } \mu$, the size of foot and mouth virus, the smallest of the group, up to just within the limits of microscopic resolution or $200\text{--}250\text{m } \mu$ ($0.2\text{--}0.25 \mu$). One of the largest of the viruses is psittacosis.

(2) *Filterability* In virtue of their small dimensions these viruses are enabled to traverse filters which retain the ordinary bacteria. This fact is made use of in separating viruses from contaminating bacteria, filtration is an essential portion of virus technique. However, filterability has not been demonstrated in the case of every member of the virus group, and the nearer the viruses approach the cultivable bacteria in size the more difficult it becomes to effect a separation by means of filters.

(3) *Staining reactions and microscopy* The ordinary staining methods are of no use for the demonstration of those viruses which come within the range of visibility with the microscope. They can, however, be stained with carbol fuchsin following treatment with a mordant or by prolonged staining with Giemsa. The virus particles appear as minute cocci occurring singly, in pairs and short chains. It has been recognised for some time now that minute coccus like bodies could be demonstrated in smears of virulent material in the case of certain viruses. Borrel demonstrated this in the case of fowlpox in 1904, and Paschen in the case of variola and vaccinia in 1906. Von Prowazek suggested the name of elementary bodies for these virus particles, and the name is still retained, though the elementary bodies of fowlpox and of variola vaccinia are also spoken of as the Borrel and Paschen bodies respectively. Recent work with the virus of psittacosis, fowlpox, vaccinia, varicella and ectromelia (a disease of mice) leaves little doubt that the elementary bodies are the virus. The staining methods employed for the demonstration of elementary bodies result in the virus particle being heavily coated with the dye, and thus increased in size and rendered more readily visible. Although valuable, these methods are of no use for obtaining

virtual images of the elementary bodies. For this purpose it is necessary to examine the unstained bodies by means of light of short wave length. Barnard has evolved a technique which makes use of ultra violet light and a special dark ground condenser and by this means has obtained much valuable information respecting the elementary bodies.

(4) *Cultivation* With the exception of the virus of bovine pleuro pneumonia and possibly also vaccinia it has not been found possible to grow viruses apart from living cells. Many of them have been cultivated in tissue culture and even surviving cells in a simple saline medium may be sufficient to support growth. Vaccinia for instance may be grown satisfactorily in a medium consisting of Tyrode's solution and minced chick embryo distributed in a shallow layer in flasks of appropriate size and shape.

(5) *Tissue reactions to viruses* The reactions on the part of the host's tissues to invasion by a virus differ in some respects from those evoked by a cultivable bacterium and there is one cellular change—the inclusion body—which would appear peculiar to the virus group. Evidence suggests that the great majority of pathogenic viruses—we know little or nothing of saprophytic viruses—are incapable of multiplying outside living cells. The reaction of the cell to this invasion may be one of two kinds. Either the cell is killed or it hypertrophies and is stimulated to proliferate. Thus we may get necrosis or tissue overgrowth as the result of a virus infection. The type of lesion will depend on which of these two changes predominates and this will depend on a variety of factors such as the quantity and virulence of the invading virus and the resistance of the invaded cell. However there is a tendency of certain viruses to produce one type of change to the exclusion of the other. Yellow fever virus for instance produces essentially necrotic lesions whilst the viruses of warts and *molluscum contagiosum* give rise to a hypertrophic lesion. Inflammatory reactions commonly occur and unless the virus infection is complicated by secondary bacterial invasion these are usually characterised by an infiltration with mononuclear cells. It is probable that the inflammatory reaction is secondary to changes set up in the infected cells.

Inclusion bodies are amorphous masses of varying size which stain with acid dyes such as eosin acid fuchsin or phloxin. They may occur either in the cytoplasm or the

nucleus, and in fixed material are separated from the cellular material by a clear space or halo. Some viruses, such as vaccinia, fowlpox, rabies and ectromelia, give rise to cytoplasmic inclusions, others like herpes zoster and varicella produce nuclear inclusions, whilst both nuclear and cytoplasmic inclusions occur with variola virus. Not every member of the virus group has been shown to produce inclusion bodies but it has not been possible to reproduce them experimentally by any other means than infection with a virus. It has been shown that the cytoplasmic inclusions of vaccinia fowlpox and ectromelia consist of masses of elementary bodies embedded in a gelatinous matrix virus colonies in other words. Whether the same holds true of nuclear inclusions is not known.

The nature of filterable viruses. The various hypotheses which have been put forward to explain the nature of filterable viruses fall into two main groups —

A Those which consider that they are unorganised non living agents

B Those which look upon them as living things

According to the former view the diseased cells in virtue of some derangement of their metabolic functions produce an agent, which in turn is capable of giving rise to similar pathological changes in normal cells. The virus thus becomes both the cause and the result of the disease. The particulate nature of viruses is explained on the assumption that the unorganised agent is adsorbed on to particles produced by the affected cells. The hypotheses belonging to the second category are three in number. According to one, the viruses are the filterable phase of cultivable bacteria the second consider them to be minute bacteria, whilst the third would separate them from the bacteria as representatives of an even simpler form of living matter. There is practically no evidence in support of the first of these hypotheses, and it would seem almost impossible to decide which of the remaining two is correct. It is doubtful even, whether it is a decision which it is of great importance to take. The important question is whether or not these viruses are living things, and since they possess the attributes of life, viz., power to multiply, ability to adapt themselves to altered environment and the power of retaining their identity regardless of environment, one is entitled to assume that they are alive.

Immunity to viruses. Recovery from infection with a virus

is usually associated with the development of a solid and lasting immunity. It is well recognised that an individual rarely contracts such diseases as measles mumps varicella smallpox or yellow fever more than once. Exceptions to this statement are the common cold herpes and influenza which leave behind them little or no immunity. It has been recognised for some time now that virus infections called forth the appearance of specific antibodies in the blood but until comparatively recently there was a tendency to look on these antibodies as different from the bacterial antibodies and to minimise the role played by them in ensuring the immune state. Immunity to a virus was considered to be a tissue immunity and something differing fundamentally from bacterial immunity. There seems now to be a fairly general agreement that no such fundamental difference exists. The differences that do occur are apparent rather than real and depend on the difference in size and mode of life of these two classes of disease agents. As an example of this one might cite the inefficacy of serum therapy in contradistinction to serum prophylaxis in virus infections which is due to the intracellular mode of life of these agents once entrenched inside a cell they are protected against the action of circulating antibodies.

Active immunity Two classical examples of artificial active immunisation against infection belong to the realm of virus diseases viz vaccination against smallpox and the method of inoculation against rabies evolved by Pasteur. In the former one makes use of living smallpox virus which has been adapted to growth in the cow and has lost in consequence its virulence for man. This altered smallpox virus is spoken of as vaccinia virus. In rabies prophylaxis the vaccine is made from virulent rabbits cord the virus contained therein being attenuated by drying over sulphuric acid for varying lengths of time (Pasteurian method) or by treating with some chemical such as carbolic acid. The incubation period of rabies being a long one there is time to immunise the individual after infection has occurred and so prevent the disease. Both these methods of immunisation have encephalomyelitis as a rare but serious sequela. This encephalomyelitis is characterised histologically by a perivascular demyelination similar to that seen in the post infection encephalitis occurring rarely as a complication of measles smallpox and varicella. The aetiology is obscure.

Experiments have shown that viruses which have been inactivated by heat have lost their power of immunising whereas inactivation by low concentrations of formalin preserves much of their antigenic value. Though the immunity produced by formalised virus is not as good as that evoked by infection with living virus it is sufficiently good to allow of living vaccines to be used subsequently without danger. This method of vaccinating first with formalised virus and then after an interval with living virus gives a solid immunity it is used in practice in protecting young dogs against distemper. Another method of active immunisation against a virus is the simultaneous inoculation of active virus and specific antiserum either mixed together or separately in different parts of the animal. A method on these lines has been evolved for the prophylactic inoculation against yellow fever it has been used for the protection of laboratory workers against this highly contagious and deadly disease.

Passive immunity In the case of many viruses it has been found possible to confer passive immunity on a normal animal by introducing an adequate dose of serum from an animal possessing active immunity. To be effective the antiserum must reach the susceptible tissues before the virus given later antiviral sera are valueless. Antiviral sera are made use of in two human diseases—measles and poliomyelitis. In the case of measles human convalescent serum is used and complete or partial protection can be given according to the interval which has been allowed to elapse between exposure to infection and administration of serum. Given during the first 5 days after exposure complete protection is obtained whereas given from the sixth to ninth day the serum modifies but does not prevent the disease after the ninth day it has no effect. The dose employed is 5 c c for those under 5 years of age and 10 c c for those over that age. Normal adult serum has been found effective but if this is used the dosage should be doubled. In poliomyelitis antiserum is given early—in the preparalytic stage—with a view to reducing the severity of the disease. Convalescent serum and the serum of adults who have had the disease are usually employed though limited supplies of antipoliomyelitis serum made in the horse are now available the latter has not yet had adequate trial. Evidence suggests that given early convalescent serum materially reduces the chance of residual paralysis and lowers the mortality.

rate Opinion differs as to dosage Doses varying from 20 c.c. intramuscularly to 100 c.c. divided between the thecal and venous routes have been advocated The making of a diagnosis early enough for the administration of serum to be worth while can only be hoped for in times of epidemics Convalescent varicella serum has been used as a prophylactic but the results obtained have not been as successful as in measles due possibly to a greater variation in potency of batches of varicella convalescent serum Somewhat similar results have been obtained in mumps but both in varicella and mumps the method is worth trying where it is required to prevent or modify the disease in susceptible individuals who have been exposed to infection

Serological reactions Just as in the case of the ordinary bacteria the interaction between virus and specific antibody can be demonstrated by a variety of reactions in the test tube The reactions of complement fixation and precipitation are both available for this purpose and where the virus is of sufficient size agglutination of the elementary bodies can be carried out The neutralisation reaction is another means of demonstrating this specific interaction but for this test a susceptible animal is necessary The virus and antiserum are mixed in appropriate doses and after a period of contact injected into a susceptible animal The absence of reaction to this inoculation indicates that the virus and antiserum are specific for one another Although these serological reactions are not as yet in current use their practical value as a means to diagnosis has been demonstrated in some of the virus diseases

RICKETTSIÆ

The Rickettsiæ so called in honour of Ricketts who described the first species the causal agent of Rocky Mountain spotted fever are small bacterium like micro-organisms which are primarily inhabitants of the alimentary canal of arthropods Some however, have become adapted to an alternate existence in insects and mammals though this alternation in hosts does not entail separate developmental cycles as in the case of so many protozoa

Rickettsial diseases of man Four diseases of man have been shown to be due to organisms belonging to this group typhus fever (*R. prowazeki* named after Ricketts and von Prowazek

who both lost their lives studying this disease) trench fever (*R. quintana* syn *R. wolhynica*) Rocky Mountain spotted fever (*R. rickettsi* syn *Dermacentrolexenus rickettsi*) and tsutsugamushi fever of Japan (*R. nipponica*) Trench fever came and went with the war and even during the war only rare cases occurred in this country it was louse borne Typhus fever occurs in two forms epidemic typhus which is highly contagious and is associated with poverty squalor and over crowding and sporadic endemic typhus which is generally less severe and lacks the contagiousness of the epidemic form It would appear that both types are due to the same causal agent but the epidemic form is transmitted by the body louse and has man as the reservoir of virus whereas the sporadic type is probably a disease of various species of rodents and is transmitted by acarines the infection of man being merely incidental this difference in epidemiology accounts for the difference presented by the two types of the disease The epidemic form of typhus alone occurs in this country At one time prevalent it is becoming increasingly rare and apart from small outbreaks in Ireland and more rarely in Liverpool and Glasgow it has not been seen in Great Britain this century The other two diseases Rocky Mountain spotted fever and tsutsugamushi fever are both typhus like diseases which like sporadic typhus remain endemic and never assume epidemic proportions They are both probably diseases of rodents transmitted by insects the wood tick *Dermacentor venustus* in the case of Rocky Mountain spotted fever and a mite the larva of *Trombicula akamushi* in tsutsugamushi fever

Morphology and staining *Rickettsiæ* present the appearance of minute cocci diplococci or short bacilli They are $0.3-0.5\ \mu$ long by $0.3\ \mu$ broad though occasional bacillary forms may extend up to 1.5 or $2\ \mu$ in length They stain poorly with the ordinary aniline dyes but can be readily demonstrated after prolonged staining with Giemsa or by means of Castaneda's stain Bipolar staining is frequently seen They are Gram negative

To stain

Fix smear in methyl alcohol for 5 minutes

Apply Castaneda's stain (p 254) and allow to act for 2 to 5 minutes

Wash

Stain with aqueous safranin for 10 seconds

Wash and blot dry

Rickettsiæ are stained blue, the cells and cell debris stained red with the safranin. This method stains some of the viruses *e.g.*, psittacosis, satisfactorily.

Cultivation With the possible exception of *R. nipponica* none of the rickettsiæ pathogenic for man have been cultivated on artificial media. Like the filterable viruses, they can be grown *in vitro* in conjunction with living cells.

Serology The serum of typhus convalescents contains specific antibody, as can be shown, for instance, by agglutination of suspensions of *R. prowazeki*. But the most interesting feature of the serology of typhus fever is the power possessed by these sera of agglutinating certain strains of *B. proteus*— λ 19 in particular—an observation made by Weil and Felix. The Weil Felix reaction is positive in all forms of typhus, epidemic and sporadic, though in the case of the sporadic rural typhus of the Malay Peninsula it is a variant of proteus λ 19—the “Kingsbury” strain—which is agglutinated, the reaction being negative with λ 19. The Weil Felix reaction is given by cases of Rocky Mountain spotted fever, but not by trench fever or tsutsugamushi fever. The reaction is of the greatest value in the diagnosis of typhus fever.

Nature of Rickettsiæ The view that Rickettsiæ are living micro-organisms is now held by the majority. In an endeavour to account for the constant presence in high titre of agglutinins for proteus λ 19 in typhus fever sera, the view has been put forward that *R. prowazeki* is merely a developmental stage of *B. proteus*. According to this view, the proteus bacillus would be the vegetative phase of the typhus virus and the rickettsia the virulent phase. Another view is that the rickettsia is the result of the action of a bacteriophage on *B. proteus* λ 19. Evidence has been advanced in support of both of these hypotheses but proof of either is lacking. The recent work of Castaneda and Zia, showing that *R. prowazeki* and *B. proteus* λ 19 possess a common antigen in addition to their specific antigens, provides an adequate explanation of the Weil Felix reaction without making it necessary to assume any genetic relationship between the proteus bacillus and the rickettsia.

CHAPTER VIII

FUNGI—PROTOZOA—HELMINTHES—ARTHROPODA

FUNGI

THE fungi are typically composed of a mycelium and spores, the latter being formed either as the result of a sexual process or by a kind of separation from the mycelium. Numerous affections, particularly of the skin in man are unquestionably the result of fungus invasion. In other conditions the part played by the fungi which may be present in the lesions is difficult to assess. An account of all the fungi which have been credited with pathogenicity to man is beyond the scope of this book, and only the more important and commoner parasites are briefly considered here.

Method. In the examination of skin lesions or hairs the following method is simple and satisfactory both for microscopy and culture.—Take scrapings from the lesion into a sterile watch glass. Put a few fragments on a slide and add a drop of sodium sulphide solution. Put under a cover slip and examine unstained. Mycelium and spores stand out clearly. The sodium sulphide solution is made by taking a few drops of saturated sodium sulphide in a test tube adding an equal volume of methylated spirit and shaking. Distilled water is then added drop by drop until the solution becomes clear. In place of the sodium sulphide solution 10 per cent caustic soda or potash may be used. For culture a fragment is placed between two filter papers and the papers are moistened with methylated spirit. The fragment is then removed with a platinum wire, embedded in a tube of Sabouraud's medium (p. 251) and incubated at room temperature.

The following are among the more important pathogenic fungi.

Microsporum. The commonest member is *M. Audouinii*, which is peculiar to the human race, and produces both scalp and body ringworm. In the hair the branching mycelial filaments break up into small spores which form a mosaic

around the shaft. There is no arrangement in chains. Similar fungi of animal—*M. felineum*, *M. lanosum* (canis) and *M. equinum*—may also affect man and infections by the cat ring worm are not uncommon. Scales taken from the skin in an infection by *M. felineum* show long thin filaments but no spores. *Microsporum furfur* is the cause of the discoloration of the skin known as pityriasis versicolor. The spores and interlacing mycelium can be easily demonstrated in scraping from the skin.

Endothrix (*Trichophyton endothrix*). The numerous varieties recorded in this group are said to be peculiar to man. They cause ringworm of the scalp, body and beard and are the most frequent species found infecting the nail plate. The spores are slightly larger than those of the microsporum group, are arranged in chains and in established cases only within the hair shaft. The mycelium is scanty, rod-like and non-septate.

Ectothrix (*Trichophyton ectothrix*). Infections by fungi of this group are derived from animals and produce ringworm of the skin, scalp and beard. Under the microscope the hair shaft is surrounded by a dense system of winding chains of spores.

Epidermophyton inguinale is the cause of *tinea cruris*. Scrapings from the skin show mycelial threads and interlacing clumps of spores.

Favus. The organisms in this group differ from other fungi in their method of growth. The mycelial threads grow out vertically instead of horizontally and the spores are found on their superficial aspect. Small crateriform lesions are produced in man on the scalp, the skin and less commonly the nail. *Achorion Schœnleini* is the favus organism most often found in man and *Achorion Quinckeianum*, a parasite of the mouse, also affects man.

Sporotrichum. A chronic infective granulomatous condition of the skin resembling in its clinical features tuberculosis or syphilis is associated with a yeast-like organism, the *Sporotrichum beurmanni*. The oval Gram-positive spores are present in the pus and in cultures taken on maltose agar and grown at room temperature, both spores and branching mycelium are found. Agglutinins are produced to the spores.

Monilia. *M. Albicans* is the principal agent of thrush. It grows like a yeast in culture, showing large oval budding cells and in scrapings from the throat coarse mycelial threads are seen together with, as a rule, the yeast-like cell.

Aspergillus A large number of this species have been described in human pathology and particularly in connection with *mycetoma* or Madura foot. The fungi have also been isolated from the sputum in pulmonary affections from the air sinuses of the skull and from the auditory meatus. In the last situation they may be associated with a pigmented granulomatous condition.

The fungi hitherto mentioned can all be easily recognised as fungi in microscopic preparations. The presence of spores and mycelial threads and in hairs the relationship of the organism to the hair shaft are usually obvious. A long and constant experience of these parasites will enable an expert to identify them under the microscope with some success. Further information can usually be obtained by cultivation. The organisms will usually grow well on ordinary media such as agar but the appearances upon which identification rests vary with the media used and it is advisable to compare all cultural growth on the same media preferably Sabouraud's medium which is in common use. On this medium the colonies may be humid and glutinous as in the case of *A. Schænleini* or snowy white downy discs as with *M. Audouinii* or pigmented as with some of the animal ringworms. In the majority of the above infections met with in practice the clinical and microscopic pictures combined where possible with the appearance of the colony on Sabouraud's medium will suffice for a diagnosis. Absolute identification and classification in this vast field is the sphere of the mycologist and owing to the difference of his outlook the result will probably have little meaning to the dermatologist.

Actinomyces The parasitology of the disease actinomycosis in man and cattle has been obscured by the discovery of at least three different organisms in the lesions. These are *Actinomyces bovis* Harz an anaerobe and the cause of actinomycosis in man and cattle. *Actinomyces graminis* Bostroem aerobic saprophytic on grains and grasses non pathogenic but occasionally isolated as a contaminant in actinomycotic lesions. The third organism is a non branching Gram negative bacillus (*actinobacillus*) which is responsible in cattle for actinobacillosis a condition resembling and often confused with actinomycosis. In addition other actinomyces are recognised including the aerobic *Actinomyces maduræ* the cause of the pale variety of Madura foot in man and *Actinomyces*

farcinus, an aerobic acid fast organism producing farcy bud in cattle

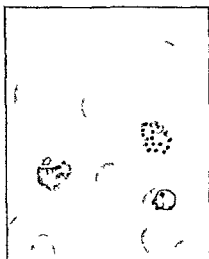
Actinomyces bovis is the causative organism of actinomycosis in man, producing lesions about the mouth or in the alimentary tract and in the lung. The pus from a discharging sinus contains colonies of the organism which appear as yellowish, round and usually soft granules. These granules are best detected by collecting the pus in a test tube, which is sloped and rotated so as to let the pus flow round the sides of the tube, this is then returned to the vertical position, and as the pus collects again at the bottom the granules can easily be seen still adhering to the sides. A granule is picked out on a platinum loop and rubbed (or, if an old and brittle granule, crushed) on a slide. The film is stained by Gram's method, and consists mainly of a delicate, Gram positive, beaded and branching mycelium (Plate VIII). Short Gram positive rods and coccoid bodies resulting from the breaking down of the mycelium may also be seen. The Gram negative clubs, which form so striking an appearance in histological sections and are deposits formed around the mycelial extremities, are rarely identified in film preparations nor do they commonly form in cultures. In section the clubs may be shown to be very feebly acid fast. In anaerobic culture at 37° C the organism grows on the ordinary media, and in broth produces white, mulberry like granules which sink to the bottom of the tube. On agar the colonies are dull white with a cauliflower like surface. Inoculation of cultures into rabbits and guinea pigs produces small non progressive lesions.

The actino-bacillus is a short Gram negative bacillus which grows upon the ordinary media. It is the chief agent in the granulomatous disease of cattle associated with "woody" tongue, a condition less commonly produced by actinomyces. The bacillus does not seem to affect man.

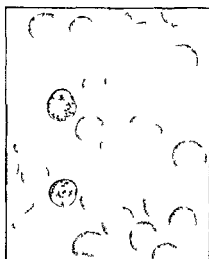
PROTOZOA

Malaria. Ague is now practically non-existent in England although it was until comparative recently endemic in the fen districts, and members of the *anopheline*, the mosquitoes responsible for the spread of the disease, are still to be found there. During and shortly after the war sporadic cases occurred in which returned soldiers with malaria acted as reservoirs.

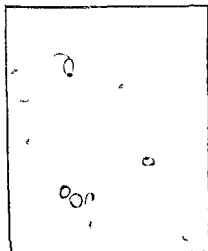
PLATE V



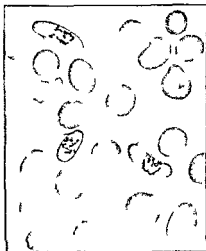
Benign Tertiar
Plasmodium vivax



Quaternary
(Plasmodium malariae)



Subtertian Rings
(Plasmodium falciparum)



Subtertian Crescents

MALAYIA PARASITES
(Leitch's Station)

Those who have lived in malarial countries, on their return to England, are apt to look upon any febrile condition as malarial, and it indeed appears that many who have been infected over long periods are subsequently liable to considerable rises of temperature from comparatively trifling causes. Cases of refractive infection by the benign tertian and quartan parasites may carry the organism for many months and even years, after leaving a malarial district and patients recently returned from infected countries may harbour the parasites in considerable numbers. In all suspected cases it is advisable to withhold quinine until the blood has been examined, in order that the diagnosis may be confirmed and the type of organism determined.

The malarial parasites are three in number the benign tertian (*Plasmodium vivax*), the quartan (*Plasmodium malariae*), and the subtertian (*Plasmodium falciparum*). The parasites all pass through very similar developmental cycles. The intracorporeal cycle (of 48 to 72 hours according to the species) commences with the appearance of an intra corpuscular disc containing scattered pigment or hæmozoin. The pigment collects into a central mass around which the parasite becomes arranged in segments or spores. The red corpuscle containing the spores breaks down and the parasites enter new corpuscles, exhibit amœboid movement, become enlarged and later again segmented. Outside the corpuscle the gametocytes derived from the freed sexual cells are in the benign tertian and quartan round in the subtertian crescentic. The crescentic male gametocyte has scattered pigment, in the female the pigment is collected in a ring in the centre of the parasite. The male gametocyte of all species becomes spherical and flagellated, the flagella, which are known as microgametes, break away and enter the spherical and quiescent female gametocytes, which after impregnation are called zygotes. The zygote, after ingestion by a mosquito, becomes elongated and motile, penetrates into the wall of the mosquito's stomach and becomes encysted. Within the cyst spindles or sporozoites are developed, and these pass to the salivary glands of the mosquito, and are thus transferred to the human host. Owing to the frequency of mixed infections the temperature chart is not as a rule a sufficient guide to the nature of the organism, which must be identified by means of the microscope (Plate V). The quartan and the benign tertian forms sporulate in the peripheral

circulation, and are the two parasites most easily confused. The quartan is feebly amœboid, its pigment granules are coarse, and the parasite commonly fills the red cell without distending it. The rosette contains 8 to 10 segments. The benign tertian is the parasite most commonly met with; it is actively amœboid and contains fine pigment granules; it only partially fills the red cell, which is almost always enlarged, frequently shows polychromatophilic degeneration and commonly contains numerous chromophilic granules known as Schuffner's dots. The rosette contains 15 to 26 segments. The fresh blood should always be examined in order to observe the activity of the parasite and the dancing movements of the pigment granules. It is advisable to use a $\frac{1}{12}$ -inch objective, and if the blood is examined immediately a warm stage is unnecessary. The rosette forms are not commonly met with in this country. The great majority of the parasites in a preparation made with Leishman's stain show an irregularly-shaped blue body containing a small knot of purple staining chromatin and black pigment granules. The malignant tertian parasite is a more serious infection, responds less readily to quinine, is frequently associated with a very grave anaemia, and may be complicated by blackwater fever. This parasite sporulates in the internal organs, and the forms present in the peripheral circulation are readily distinguished, since the commonest appearances met with are the so-called signet rings and crescents. In Leishman stained preparations the 'signets' show as small delicate blue rings with a knot of purple chromatin at one spot on their circumference. The crescents are blue crescentic bodies containing the typical black pigment granules. The crescents appear at first sight to be lying free in the blood, but on closer inspection a narrow rim of the cytoplasm of the red blood corpuscle can be made out, often bridging the concavity of the crescent. The malignant tertian parasites are commonly very scanty and may require a prolonged search before they can be demonstrated.

The best method of looking for all forms of malarial parasites is to make films in the ordinary way, and stain them by Leishman's stain. A prolonged search may be necessary, and if the parasites cannot be found by the usual methods it is advisable to make the films as thick as possible, and when dry to hæmolyse them by dipping them in tap water until

no more hæmoglobin comes out. They should then be stained in carbol thionin for 3 minutes, washed in water and blotted dry.

Trypanosomiasis. Trypanosome infections in man are rarely met with and never arise in this country, but the organisms are so widely spread and so fatal to man and animals that a very short account of them may be given here. Trypanosomes are to be found in the blood of a large variety of animals, and in many of them appear to produce no ill effect, while in others they cause disease and often a heavy mortality. In man trypanosomiasis is the cause of sleeping sickness, a disease which has almost depopulated vast areas of country in Africa, and of Chagas' disease in South America.

The more important trypanosomes are the following —

T. lewisi, the rat trypanosome

T. evansi, which attacks camels, elephants, etc., and is the cause of the disease known in India as "Surra."

T. brucei, which attacks horses and bovines and produces the disease called "Nagana" in Africa. This parasite is found also in the native antelopes, which appear to be immune to its poison and to act as reservoirs for the infection of the domestic animals. *T. brucei* is spread by a tsetse fly, *Glossina morsitans*.

T. gambiense is the chief cause of sleeping sickness in man, and is spread by another biting fly, *Glossina palpalis*. *T. rhodesiense* is less widely distributed, produces a more virulent form of sleeping sickness, and is spread by *Glossina morsitans*. In the early stages of the disease the trypanosomes are present in the blood and more numerous in the lymphatic glands, and may then cause few symptoms. It is only in the late stages that the organisms gain access to the central nervous system, are found in the cerebro spinal fluid, and produce the symptoms of the disease.

In order to demonstrate the presence of the trypanosomes in a suspected case the blood should be examined both in the fresh state and in ordinary films stained by Leishman's stain. The parasite is, however, frequently scanty in the peripheral circulation, and it may be necessary to withdraw several cubic centimetres of the blood from a vein, mix them in citrated salt solution, centrifuge, and examine the deposit. Usually the lymphatic glands are enlarged, and most frequently the cervical glands, and the simplest method is to puncture the

most prominent gland with a hypodermic needle and syringe and make films from the small quantity of fluid obtainable. By this method trypanosomes can usually be demonstrated with ease. In the later stages the cerebro spinal fluid may be removed by lumbar puncture centrifuged and the deposit examined. An excess of lymphocytes is present in the fluid in addition to the parasites themselves. If parasites cannot be found by any of these methods the conclusive proof of absence of infection rests upon the inoculation of susceptible animals with the patient's blood.

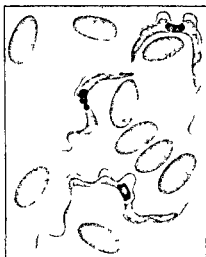
Chagas disease of South America is caused by the *trypanosoma cruzi* and is disseminated by bugs, the armadillo and the opossum acting as reservoirs.

A trypanosome as seen in the fresh blood is actively motile and provided with a free flagellum at its anterior extremity. In stained preparations the following points may be made out (Plate VI). Near the posterior rounded extremity is a small deeply staining round spot the blepharoblast. The nucleus is commonly situated near the centre of the trypanosome. The undulating membrane can be seen arising from the blepharoblast winding along the free border of the parasite and terminating in the flagellum at the posterior extremity. The identification of the different species of trypanosomes must be left to the expert.

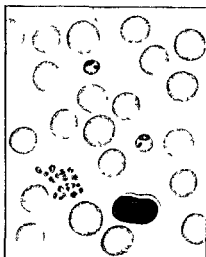
The trypanosomes can be cultivated in special agar blood media the forms pathogenic to man being more difficult to culture than the non pathogenic species or those found in birds.

Leishmaniasis includes a generalised infection called in India kala azar and a local condition known as Oriental or tropical sore. Kala azar may be epidemic as in Assam or sporadic as in the Mediterranean basin where it is mainly confined to young children. In addition there is a naso pharyngeal form of Leishmaniasis met with in South America and known as espundia. In all these conditions the infecting protozoon is a small circular body containing a round nucleus and a small deeply staining rod-shaped micro nucleus the organism in this stage of its cycle being known as the Leishman Donovan body (Plate VI). In Oriental sore the parasites can be demonstrated in scrapings from the lesion and in kala azar they may be searched for in the blood in which they may be found within the leucocytes and usually the large hyaline cells. The

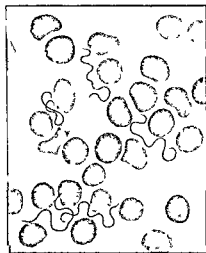
PLATE VI.



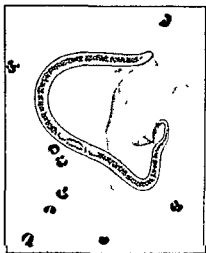
Trypanosomes.
(Camel's Blood (Leishman's Stain))



Leishman-Donovan Bodies
(Leishman's Stain)



Spirilla of Relapsing Fever
(Mouse's Blood)
(Leishman's Stain)



Filaria and Sheath
Hemolyzed Blood Film
(Hematoxylin)

parasites however are commonly scanty in the peripheral circulation and it may not be possible to find them even after a prolonged search. The most practical method is to perform puncture of the spleen a proceeding which has in the past been attended by fatal results owing to the wounding of considerable blood vessels with a large needle. If puncture of the spleen is performed with the ordinary hypodermic syringe armed with the usual fine needle the operation is practically devoid of risk and the minute quantity of splenic fluid obtained is quite sufficient for diagnostic purposes. When performing splenic puncture the patient must be made to take a deep breath and hold it. Films made from the fluid should be stained with Leishman's stain and the numerous distinctive parasites will be found both lying free and massed within the endothelial cells of the spleen. The organisms can be cultivated in citrated blood at 20° C. when they develop into elongated motile flagellated forms which resemble trypanosomes but have no undulating membrane. The full story of the transmission of the parasites is not yet known but it is probable that they are abstracted from the peripheral blood or from beneath the skin by biting insects and it is almost certain that sandflies can thus transmit the infection. Natural reservoirs are provided by the dog in some areas and a small rodent the hamster in others.

Amœbiasis. Amœbic dysentery is a disease of tropical and subtropical countries but occasional cases have been reported in patients who have never been out of Great Britain. The causative organism the *Entamœba histolytica* is abundant in the stools in the acute stage. In the chronic stage in convalescents and in contacts who have never had the disease the organisms in their amœbic or cystic forms may be found in the stools but are often scanty and other non pathogenic amœbæ may be present. In the freshly evacuated pus of a liver abscess amœbæ can rarely be detected but they may be abundant in the later discharge. Of the non pathogenic amœbæ the *Entamœba coli* is most commonly met with and the chief points of distinction between this organism and *E. histolytica* are the following. *E. histolytica* moves more actively with clear pseudopodia frequently ingests red cells has a granular endoplasm and clear ectoplasm. *E. coli* is more sluggish ingests bacteria but not red cells has no distinction between ecto and endo plasm and in fresh specimens has a

distinct nucleus that of *E histolytica* being hard to see. The cysts of *E coli* are larger than those of *E histolytica* and have more nuclei which reach to 8 in the former and to 4 in the latter. Coarse chromatoid bodies are often present in cysts of *E histolytica* but not in those of *E coli*. Certain identification of the different species of amœbæ is often difficult except with constant practice but it should be possible to distinguish actively moving amœbæ or spherical nucleated cysts from intestinal epithelial phagocytic cells or the more or less circular non nucleated or mono nuclear objects commonly encountered in the feces. The stool should always be examined within an hour or two of being passed and emetine should be withheld until the examination has been made. A drop of blood stained mucus is selected placed on a slide with a cover glass over it and examined with a $\frac{1}{8}$ inch objective. A warm stage is not essential but the amœbic movements are more certainly seen by this method. On a cold day the ordinary slide preparation should be warmed and in any case it should be examined immediately after it has been made. If the amœbæ are few in number proceed as follows. Place a shred of mucus on each of two slides and mix in saline on one slide and in triple-strength Gram's iodine on the other slide. Examine with low power objectives preferably on a warm stage and confirm under a $\frac{1}{4}$ inch objective. The chromatoid bodies are deeply stained by the iodine.

Al o make a film of undiluted feces on a slide with a platinum loop. Place without drying in Zenker's fluid 5 to 10 minutes then into 80 per cent alcohol 10 to 20 minutes. Then without drying stain with Van Gieson's method of section staining. Examine for amœbæ and cysts.

Flagellates Numerous flagellates have been found to occur often in considerable numbers in human feces. Of these *Lambia intestinalis* (*Giardia intestinalis*) has caused considerable controversy. It measures 10 by 15 μ and has a pear-shaped body with two oval nuclei and four pairs of flagella. The balance of opinion would seem to be that the e parasites are devoid of pathogenic action.

Trichomonas of more than one variety may be present in large numbers in the stools. The bodies are pear-shaped a single nucleus is present and three four or five flagella. Cysts of this protozoon and of *Lambia intestinalis* may be numerous.

Infusoria The *Balantidium coli* is the only important

protozoon of this class which is credited with pathogenic action in man, infections having been recorded on the Continent, in the Philippines and elsewhere. The organism is a large oval parasite covered with cilia; the cysts are slightly ovoid and from 50 to 60 μ in diameter.

HELMINTHES

A considerable variety of animal parasites may on certain occasions and in certain countries infest the human alimentary tract. Only three species are however, commonly distributed in this country—namely, *Oxyuris vermicularis*, *Ascaris lumbricoides* and *Tænia saginata*. Other species, such as *ancylostoma*, are common in certain circumscribed areas. Others again may be imported by their human host from other countries, or may occur only fortuitously in man.

The following is a brief account of the more important parasites, arranged zoologically.

Platyhelminthes. These are worm like, flattened dorso-ventrally and frequently hermaphroditic.

They are divided into three classes—

Class 1, Turbellaria are not parasitic.

Class 2, Trematoda (or flukes), are parasitic in man. They are unsegmented. In all the life cycle from ovum to adult is complex requiring an intermediate host, and asexual multiplication takes place outside the body from the sexually-produced egg.

Class 3, Cestoda (tape worms), are parasitic, are elongated, flattened, and segmented, the mode of development is comparatively simple.

Trematoda. A considerable number of flukes are parasitic to man, but none which produce symptoms are native to this country. The flukes may conveniently be divided on clinical grounds into those which inhabit the biliary passages, the bronchi, and the blood vessels.

Liver flukes are numerous, the most important being *Fasciolopsis buski*, common in China and India, and *Clonorchis sinensis*, an extremely frequent parasite of Eastern peoples. Usually no symptoms are produced, but there may be enlargement of the liver with jaundice and ascites. The ova are found in the faeces. They are roughly oval, but with one end narrower than the other, and are of a yellowish colour. They

have a shell with a lid at one end and contain round refractile globules

The bronchial fluke or *Paragonimus westermani* is the cause of endemic hæmoptysis in China Japan and the Philip pines It measures about 10 mm long and 3 mm broad Infection is diagnosed by the ready detection of the ova in the sputum More rarely the parasite is found in the intestine and the ova in the fæces The intermediate hosts are certain fresh water molluscs and crabs The young flukes or cercariæ are taken into the mouth with the food and subsequently perforate the diaphragm and penetrating the lungs become encysted there

The blood flukes of which the most important is *Schistosoma hæmatobium* are bisexual trematodes *S hæmatobium* or *Bilharzia hæmatobia* as it was formerly called is a common



FIG. 11.—*Schistosoma hæmatobium*
Ovum Male and Female Natural Size

parasite of Egypt and parts of South Africa The adult worm lives in the veins without producing much change in them The symptoms are consequent upon the passage of the ova through the mucous membrane into the rectum or more

commonly bladder The diagnosis is determined by the presence of the spined eggs in the urine or the fæces The ova differ from those of other trematodes in having no lid The single spine is terminal and the ova may be found some years after the patient has left the infected district The eggs contain a ciliated embryo The adult male is from 15 to 18 mm long and 3 to 5 mm broad The female is longer and thinner being 20 mm long and only 0.25 mm broad The male has two suckers the anterior of which is terminal It is a flat worm rolled laterally upon itself to form a hollow tube within which the female is clasped When the ova are deposited in water the ciliated embryo escapes and forms a sporocyst in certain fresh water snails From the cyst tailed cercariæ develop escape from the snail and enter the human body by penetrating the skin or buccal mucous membrane They then pass into the portal circulation where they develop

into the adult worms. The *S. Mansoni* is a similar fluke inhabiting the capillaries of the intestine and frequently producing rectal polypi. The intermediate host is another species of fresh water snail. The ova are found in the faeces and have a lateral instead of a terminal spine.

A smaller trematode also inhabiting the portal veins of man and common in the East is *S. japonicum*. The male similarly contains the female in a gynæcophoric canal. The ova are found in the faeces and contain a ciliated embryo but have no spine.

Cestoda The tape worms are divided into two groups by the characters of their heads namely *Pseudophyllidea* and *Cyclophyllidea*. The heads of the former are provided with two elongated slits while the latter have four round suckers and a rostellum which in some species is armed with hooklets. All the worms consist of a head or scolex from which arises a series of segments or proglottides. Each proglottis is bisexual.

Pseudophyllidea The most important human parasite of this class is *Dibothriocephalus latus*.

Dibothriocephalus latus is not met with in Great Britain but is common in Iceland, Switzerland, Russia and in parts of America. The adult worm lives in the intestinal tract of man and feeds upon the intestinal contents. The symptoms produced in the host are usually trifling but in exceptional cases a severe anæmia with a high colour index may develop. The parasite is very large and long and may grow to 25 or 30 feet in length. The head is long and narrow and is attached to the gut wall by two long slit like suckers. The genital pore opens upon the flat surface of the proglottis. The ova are enclosed in shells fitted with a lid and at the time of passage in the faeces are immature. The shells are almost colourless. Within the ovum a six hooked embryo forms with a ciliated capsule around it. Embryo and capsule are called the oncosphere. The ciliated embryo escapes in water and enters first a water flea and subsequently a freshwater fish. Here the ciliated envelope disappears and the embryo makes its way into the muscles and losing its hooklets develops slit like suckers and an unsegmented worm like body. This larval form is eaten by man or other host and develops into the adult segmented worm.

A similar and even larger tape worm is met with in Japan.

It may attain a length of 12 yards, and is known as *Diplogonoporus grandis*

Cyclophyllidea are represented by several species which may be parasitic to man, and three are common. Man is the definitive host in the case of *Tænia solium* and *Tænia saginata*, but for the former may act as the intermediate host. Man is the intermediate host for *Echinococcus granulosus* or *hydatid*. The ova of the tænidæ and of echinococcus ripen in the proglottides and at the time of passage in the fæces are in the oncosphere stage. At this stage the embryo has a head armed with six hooklets, and is contained in a thick, radially striated, but not ciliated capsule. The oncospheres are swallowed by man or sheep in the case of the *echinococcus*, by cattle with the *T. saginata*, and by pigs with the *T. solium*. The capsule is dissolved in the intestinal canal of the intermediate host, and the hooked embryo pierces the gut wall and reaches the viscera. Here the embryo becomes encysted and its hooklets drop off. The cyst or cysticercus becomes lined with germ cells, from which scolices develop. The echinococcus cysts develop secondary or daughter cysts, and the scolices grow in



FIG. 12.—Head of *Tænia solium*.
Hooklet. Head Natural Size.

these. The cysticercus is ingested by a flesh-eating animal, the scolices are set free, attach themselves to the gut wall, and grow into adult worms.

Tænia solium, the pork tape worm, reaches a length of about 10 feet. The ripe proglottis is about 10 mm long and 5 mm broad. The genital pores, as is the case in all the tænidæ, open laterally. The uterus has about 10 lateral branches. The rostellum has 4 suckers and a double circle of hooklets. Man becomes infected by eating imperfectly-cooked and "measled" pork. The infection is much commoner in Germany than in Great Britain. Man occasionally acts as the intermediate host, and becomes infected by swallowing the oncospheres, such infection may occur when human fæces are used as manure or by auto-infection.

The adult form can be recognised by the passage of the oncospheres and the segments in the fæces. The head of the

worm which is a comparatively minute object must be particularly sought for and the rostellum with its suckers and hooklets is readily recognised with a low magnification. The cyst contents of the cysticercus infection are searched for the small hooklets which are pointed slightly curved and not barbed. This cysticercus is known as the *Cysticercus cellulosæ*.

Tænia saginata is the commonest of all human tape worms. It is larger and longer than *T. solium* and may attain a length of 20 feet. The ripe proglottis is longer and broader than that of *T. solium*. The uterus has from 20 to 25 main branches. The head has 4 suckers but no hooklets and usually there is a more or less circular deposit of black pigment in the anterior part of the head. The cysticercus occurs in the muscles of the ox and is known as *Cysticercus bovis*. Man is probably never affected by the cysticercus but is always the definitive host. Persons most liable to be affected are those who eat raw or lightly cooked beef the small cysticerci in which can easily escape notice. The adult worm in the intestine produces few symptoms other than of a subjective nature. The diagnosis rests with the detection of the head the proglottides or the oncospheres in the fæces.

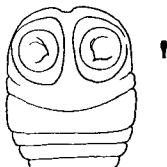


FIG. 13.—Head of *Tænia saginata*. Head Natural Size.

Echinococcus granulosus. The echinococcus or hydatid is a small tape worm only 4 or 5 mm long. It is composed of a variable number of segments usually 4 sometimes 3 or 5. The mature ova are contained in the last segment. The head is provided with 4 suckers and a double row of hooklets. The adult tape worm is found in dogs, wolves and jackals and the period between ingestion and the passage of mature ova is about 40 days. The oncospheres may be deposited on vegetables or grass and from this source man or sheep may become infected. In man the cysticercoid form only is found and the tumours may take three or more years to develop. Human infection is common in Australia and other sheep raising countries. In England it is perhaps most common in the eastern counties and a considerable number of cases are

met with in the Cambridgeshire district. Persons who have never been out of London may occasionally be infected possibly through the dangerous medium of a dog infested watercress bed. The cysticercus may be found in almost any region of the body in man and occasionally numbers of cysts may be passed in the *faeces* following the rupture of a parent cyst into the gut. The cysts may be quite small translucent round bodies not unlike grapes. Their nature is certainly determined by the finding of barbed hooklets or well formed scolices within them but it is not uncommon to find numbers of well developed but sterile cysts.



FIG. 14.—Echinococcus. Scolices and Hooklets.

Nemathelminthes. These are round unsegmented worms usually tapering at both ends.

They are divided into three classes —

Class 1 Nematoda commonly parasitic in man

Class 2 Nematomorpha not parasitic in man

Class 3 Acinθοcephala very rarely parasitic in man

Only the nematodes are considered here.

Nematoda. The nematodes include a considerable number of different families not all of which are parasitic while others are very commonly parasitic in man.

Strongyloides stercoralis is a very common intestinal parasite in tropical countries. It passes through a parasitic and a free living form. The adult parasitic form is a parthenogenic female and lives in the mucous membrane of the small intestine. It is a cylindrical worm 2.5 mm long with a pointed tail. The eggs are deposited in the intestinal mucous membrane and develop into larvæ which leave the host in the *faeces*. Here they develop further cast their skins and become sexually mature. The females are 1 mm long and the males shorter. The sexes copulate and the female lays eggs from which larvæ are hatched which develop into the parasitic

form, and cannot reproduce themselves unless again reintroduced into the human intestine

Trichuris trichiura, or the whip worm also known as *Trichocephalus dispar* is a bisexual worm with a simple developmental history The female is about 50 mm long and the male is somewhat smaller The anterior two thirds of the worm consists of a thin filiform process The posterior extremity of the male is curved upon itself and ends in a rounded projection in which the vas deferens opens The eggs are oval and have a thick brown capsule with an opening at each end The parasite is much more commonly found in the inhabitants of tropical climates than in temperate countries No ill effect is produced by the worms Infection

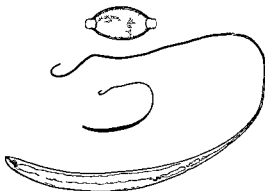


FIG 15 —*Trichuris Trichiura*
Ovum Female Natural Size

takes place by ingestion of the ova and no intermediate host is required

Trichinella spiralis is viviparous and requires two flesh eating hosts for development The females measure about 4 mm in length and the males half that size Both have a pointed anterior extremity but taper to it gradually The posterior extremity of the male has two short caudal appendages, between which the cloaca lies Trichinosis in man is rare in Great Britain, but common in Germany and America The usual host is the pig, which may become infected by eating dead rats The rats carry on the infection by eating each other Man becomes infected by eating imperfectly cooked pork The larvæ are encysted in the pork, and when swallowed are set free in the stomach They become

sexually mature in the upper part of the small intestine and copulate. Living embryos are voided into the intestine and thence travelling by the lymphatics they find their way to the striated muscle. Here they become encysted between the muscle fibres. The adult worms in the gut produce severe hæmorrhage and diarrhœa. The infection is a serious one and the migration of the larvæ into the muscles is accompanied by considerable disturbance and local pain. A well marked eosinophilia is present in the blood.

Strongylidæ By far the most important of these worms found in man is the *ancylotoma* (*uncinaria*).

Ancylostoma duodenale is a nematode worm which has a very wide distribution and causes serious symptoms. There are two distinct species of *ancylotoma* 4 *duodenale* and



FIG. 16.—*Trichinella Spiralis*
Larva Encysted in Muscle.

4 *brasiliense*. In addition a very similar parasite *Aecator Americanus* has a wide distribution but is not found in Europe and is less common in Egypt than 4 *duodenale*. In 4 *duodenale* the female is 10 to 13 mm long and the male is somewhat shorter. The anterior extremity of each is rounded and the buccal cavity is guarded by 4 incurved spines. The posterior extremity of the female is pointed while that of the male ends in a membranous expansion from which 2 long spicules protrude. The eggs are oval with a very thin transparent and colourless shell. Segmentation of the contents has commenced when they reach the faeces 2 or 4 cells being visible. In 4 *Americanus* and 4 *brasiliense* the worms are smaller and there are other minor differences. The worms are extremely widely distributed in tropical and subtropical countries but in Great Britain are confined to places where suitable climatic conditions exist for the development of the ova. The infection is found amongst workers in

the metalliferous mines of Cornwall having been introduced from foreign countries. An extremely severe anæmia accompanied by a considerable eosinophilia follows the infection. The adult worms live in the small intestine of man, being firmly attached by their heads to the mucous membrane. The ova pass out in the stools. The larvæ hatch out under favourable circumstances in about 2 days. The full grown larva is produced from the ovum in about 10 days and after moulting three or four times becomes a sexless individual moving freely in a chitinous sheath. The ova are not infective, the full grown larvæ are. Man becomes infected in one of two ways, either the larvæ are swallowed, a method which is probably

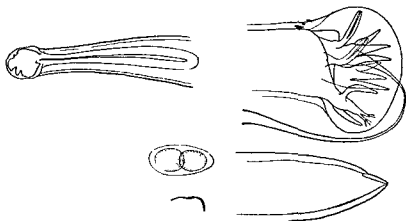


FIG. 17.—*Ancyllostomaodenale*. Anterior and Posterior Extremity of Male. Posterior Extremity of Female. Ovum. Natural Size.

the less common, or they enter through the skin, causing a local erythema or urticaria, then pass to the lungs, causing bronchial catarrh, and finally find their way to the stomach. The diagnosis is made by the finding of the ova in the feces. If the live ova are incubated they will be found to contain larvæ in about 24 hours. The adult worms are rarely seen in the feces.

Ascaris lumbricoides is the common round worm of man and has an extremely wide geographical distribution. The female is from 20 to 40 cm. long and the male about two thirds that length. The heads of both sexes have 3 prominent lips, 2 ventral and 1 dorsal. The tail of the male worm is strongly curved in a ventral direction and 2 fine spicules extrude from it. The worms are commonly found in pairs in the small

intestine and in the great majority of cases cause no symptoms. They occasionally however wander from the intestine and may make their way up the common bile duct into the gall bladder or into the liver and more rarely have been found in the pancreatic duct. The worms are only from time to time passed in the feces but the diagnosis can readily be made as a rule by finding the ova which have a characteristic appearance. The ova are fairly large measuring 0.05 by 0.07 mm and are more round than oval. The unsegmented ovum has a thick transparent colourless shell which it does not completely fill. The shell is nearly always coated with a rough granular albuminous material of a brown colour. The unfertilised ova contain refractile globules. The embryos develop in a moist atmosphere at room temperature in from 30 to 40 days and can remain alive for long periods. The life history is simple.

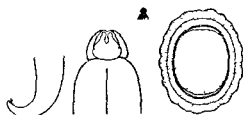


FIG 18. *Ascaris lumbricoides*. Posterior Extremity of Male. Head Enlarged and Natural Size. Ovum.

the ova containing the embryos are ingested the capsules are dissolved and the free larvæ reach maturity in the intestine in about 5 weeks.

Somewhat similar but smaller ascaridæ infest dogs and cats and are occasionally found in man.

Oxyuris vermicularis or *Enterobius vermicularis* is vulgarly known as the thread worm and is a common parasite of man and particularly of children of the poorer classes. The female is about 10 mm long and the male about half that length. The tail of the female is straight and pointed that of the male is curved and rounded. The two sexes live and copulate in the small intestine. The fertilised females leave the males and travel downwards to form large congregations in the cæcum appendix and ascending colon where they reside until the ova are nearly mature. The females then set out on their travels again pass down the rectum and appear at the anus. The ova are deposited on the mucous membrane and skin of the anus and perineum. The wanderings of the worms and the irritation of the ova and of the larvæ which may escape from them cause considerable perineal itching. The child scratches its perineum and conveys the ova to its mouth or nose or to

the face of its neighbour. The ova lose their capsules in the stomach, and the freed embryos reach maturity in about a fortnight. Fertilised females containing mature eggs are often swept out with the fæces and the diagnosis is made by detecting them in the stools. The free ova are rarely found. The ova are of about the same size and shape as those of *ancylostoma*, and are enclosed in a thin capsule. They are however, distinctly flattened on one side and at the time of passage contain a well developed embryo.

Dracunculus medinensis is a common parasite in India, Africa and South America. The female worm is thread like and some 30 inches long. The much smaller male worm has been found in the muscles and connective tissues. After fertilisation the female worm migrates and pierces the skin in the most con-

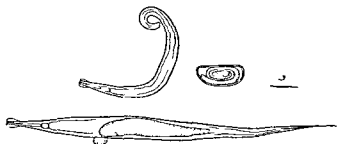


FIG 19 —*Oxyuris Vermicularis* Male Female and Natural Size
Ovum with Embryo

venient position for depositing its embryos in water. The skin is thus most commonly punctured in the neighbourhood of the foot or if the host be a water carrier the back. The embryos are ingested by a cyclops in which they undergo further development. In the larval form they measure 1 mm in length and are ingested by man finding their way to the connective tissue to complete their development into the adult form.

Filaridæ The most common embryo of the nematode worms which may be found in the blood of man is that of *F. nocturna* (*F. bancrofti*). The parent worms live in the lymphatics of the limbs or trunk and pass their young into the lymphatic stream and so into the blood. The parent worms by blocking the lymphatic circulation, may give rise to elephantiasis, or, in the rare cases in which they are located in the bladder, to chyluria. Most commonly, however, they give rise to no symptoms. The embryos appear in large numbers in

the peripheral blood at night and during the daytime retire to the heart and large vessels. The embryos in the blood are enclosed in a sheath from which they cannot escape but within which they can move. The embryos are sucked from the blood by the mosquito and in its stomach get rid of their sheath. They subsequently bore their way through the stomach wall and pass into the thoracic muscles where they undergo further development. The worm then works its way into the proboscis of the mosquito and so passes again to man. Those filaræ the embryos of which are found in the peripheral circulation of man are represented by three main species *F. nocturna*, *Loa loa* (*F. diurna*) and *Acanthocheilonema perstans*. The adults of *Loa loa* are found under the skin and about the orbit; those of *A. perstans* may be present in the mesentery, the retro-peritoneal tissues and in the pericardium. The embryos of all species should be looked for in the fresh blood and in stained preparations made from comparatively thick films. In the case of *F. nocturna* the blood should be examined between 7 o'clock at night and 7 o'clock in the morning. The embryos are easily recognised (Plate VI p. 150) and should be sought for under a low power (e.g. $\frac{2}{3}$ inch objective) of the microscope. For the differentiation of the various species the student should refer to works on tropical medicine.

Ova. For the routine examination of parasitic ova in the stools a convenient method is as follows. Make a thick emulsion of feces with a concentrated solution of sodium chloride. Fill a straight flat-bottomed tube—such as a sputum tube—to the top with the emulsion. Rest a slide on the top for five minutes. Gently remove the slide and cover with a glass. The emulsion adhering to the slide. Examine with a $\frac{2}{3}$ inch and then $\frac{1}{8}$ inch objective.

Arthropoda

Among the arthropoda the insecta, crustacea and arachnida play important parts in the dissemination of disease and many of them are referred to in the description of those infections with which they are associated. It is only possible here to give a brief account of the more common arthropoda parasitic for man.

Sarcoptes scabiei of the order *acarina* is the parasite of scabies or vulgarly itch. The diagnosis of this common condition should be confirmed by the very simple examination

needed to demonstrate the causative parasite. On the skin and usually between the fingers is seen a thin greyish black raised line about $\frac{1}{4}$ inch long forming the burrow produced by the female acarus as it travels from the surface along the skin. The burrow is dissected out with a surgical needle and at the extremity furthest from the point of entrance is found a small black speck just visible to the naked eye and evident under a hand magnifying glass as the female acarus. At intervals between the female and the surface are found the ova. The male acarus does not leave the surface of the skin and is in consequence rarely observed. The female acarus examined with a low power of the microscope is seen as a somewhat rounded oval body with eight limbs. The anterior four limbs are armed with suckers the posterior four with bristles. The male acarus is similar but smaller. The ova may be detected by dissecting out a portion of the burrow and mounting it in saline or weak potash on a slide with a cover slip over it. The oval eggs with a more or less developed contained embryo must be distinguished from the epithelial cells of the skin with their angular shape and central nucleus.

Pediculi: These parasites are remarkably particular in their habitat. Different varieties affect different parts of the human body and practically never transgress upon each other's domains. Moreover the pediculi of some animals will not pass to other species of animals for example the common body lice of dogs do not attack man. The special body lice of man are named according to their distribution *Pediculus corporis*, *Pediculus capitis* and *Pediculus pubis*.

Pediculus corporis infests the trunk and the body clothing. It is the largest of the human pediculi being about 3 mm long and readily visible to the naked eye. The ova are laid upon the hairs or the clothing. *Pediculus corporis* as is the case with the other pediculi has six legs armed with short claws. It attacks adults more commonly than children.

Pediculus capitis is confined to the hairy scalp. It has a similar shape to *Pediculus corporis* but is not so long. The numerous white ova or nits are attached to the hairs and form very conspicuous objects while the adult parasites can readily be seen on close inspection moving among the hair roots. The infection is extremely common especially among children with long hair. Adults particularly women of the lower classes are by no means exempt.

Pediculus pubis or *Phthirus pubis* is shorter and considerably stouter than either of the two former species. It is about $1\frac{1}{2}$ mm long. Its shape has earned it the euphonious name of 'crab louse'. The ova are brown and are attached to the hairs in the same manner as those of *Pediculus capitis*. Its range is almost entirely confined to the pubic hairs but the hairs of the axillæ and the eyelashes may be exceptionally infected. The spread of this parasite is usually by sexual intercourse.

Leptus autumnalis is an extremely common larva which attacks the human skin. Its vulgar name is harvest bug. In many country districts the parasite is very numerous on grass lands in July and August and attacks through thin clothing any part of the human body which may come in contact with the ground. The minute parasite buries its head in the skin. The larva is of an orange colour and 0.5 mm long. It is properly parasitic on moles and hares and dies on man in a few days. The adult form is unknown.

Demodex folliculorum. This common parasite of the hair follicles and sebaceous glands is rarely seen because of its minute size and usually harmless habits. It is from 0.3 to 0.4 mm in length, has a mouth adapted for sucking, a thorax provided with four short legs and a transversely striated abdomen. The life cycle is passed in the sebaceous ducts in any part of the skin and particularly about the face and breasts. A similar species of the dog is a cause of mange in that animal and may be transferred to man. The demodex may exceptionally multiply in the Meibomian glands and set up an intractable inflammation of the eyelids.

CHAPTER IX

IMMUNITY

RECOVERY from bacterial infection is accompanied by the development of an increased resistance to reinfection with the same bacterial species. This resistance to reinfection is spoken of as immunity. It varies both in degree and duration in the different bacterial diseases and from one individual to another in the same disease. With the onset of immunity the body fluids of the infected person can be shown to have acquired new activities which we speak of as being due to the presence of antibodies. The infecting agents which lead to the production of the antibodies are called antigens.

We have little exact knowledge of the chemical nature of antibodies but they are closely united to the serum globulins from which they cannot be separated and it is possible that each one of the very numerous antibodies which may appear in disease may derive their specific properties from a particular rearrangement of the globulin molecule. The specificity of the reactions between antigens and antibodies is precise and these reactions known as immunity reactions can be demonstrated *in vitro* by a number of methods which include precipitin agglutinin and complement deviation tests.

Agglutinins These antibodies result from bacterial infection and resemble the precipitins which appear in the serum following the parenteral injection of proteins. The reaction can be demonstrated by mixing together a saline suspension of bacteria with the serum containing its specific antibody. The bacteria run together into clumps forming larger and larger aggregates which finally fall to the bottom of the tube in the form of obvious flocculi leaving a clear supernatant fluid. Control suspensions without serum retain their original homogeneous opacity. Electrolytes are essential to this and all other immunity reactions which appear to depend on the union of antigen (bacteria) with its specific antibody and the precipitation of the resulting complex by the electrolytes. Although the antigen antibody reaction is a specific

one bacteria contain more than one antigen concerned in the phenomenon of agglutination. Many of the flagellated bacilli are known to have two such antigens or agglutinogens called the H and O agglutinogens. Of these the H antigen is derived from the flagella the O from the bacterial bodies the former giving rise to a loose diffuse clumping due to entanglement of the flagella the latter to a fine granular deposit resulting from the close packing of the bacterial bodies. There are other differences between the H and O fractions the flagellar agglutinogen being thermolabile and type specific the somatic thermostable and species specific further the somatic agglutinin appears earlier in infection than the flagellar. The reaction of the agglutinins to heat is the reverse of that of the agglutinogens the somatic agglutinin being thermolabile and the flagellar thermostable. Different bacterial strains contain the two agglutinogens in varying amounts and in the case of the typhoid bacillus a strain known as T 901 has the somatic agglutinogen only. The serum from a case of infection by any of the typhoid group will thus agglutinate T 901 at an early period of the disease but will only give the flagellar agglutination with the specific infecting organism. This occurrence in the same organism of one agglutinogen common to a bacterial group in addition to the type-specific agglutinogen may account for the Weil Felix reaction in typhus (p. 142).

The use of agglutinins in diagnosis. In making use of agglutinins in the diagnosis of disease it is insufficient to demonstrate their presence but always essential to estimate their amount and sometimes necessary to investigate their kind whether flagellar and type specific or somatic and species specific. The amount of agglutinin in any serum is judged by progressively diluting the serum until the greatest dilution capable of agglutinating the bacteria is arrived at. The application of the test is necessarily limited to those infections in which agglutinins are produced in appreciable amounts and with reasonable rapidity. For example in staphylococcal and coliform infections little or no agglutinin is commonly produced and in cholera the specific agglutinin is manifest only after recovery from the attack. In clinical pathology the agglutinin test is of particular value in the investigation of typhoid and paratyphoid fevers the bacillary dysenteries and the *Brucella* infections.

The agglutinins in typhoid and paratyphoid fevers. The

estimation of the typhoid agglutinin is known as the *Grünbaum-Widal reaction* and is of the greatest use in diagnosis in this group of fevers, the commonest infecting agent is *B typhosus* but infections with *B paratyphosus B*, and less commonly *B paratyphosus A* are often met with. Mixed infections due to one or more of the group also occur. While in civil practice in England typhoid infections are becoming progressively less frequent clinical experience of these diseases becomes limited and the necessity for laboratory aids in diagnosis remains. Distinction between the typhoid and paratyphoid infections cannot be made on clinical grounds alone. Such minor differences as the more profuse rash and the milder course of the paratyphoid fevers are matters of degree and too inconstant to be of much aid in an individual case. The reaction is not often entirely negative after the first 10 days of the fever and it is most exceptional to meet with an example of one of these infections in which the reaction remains negative throughout provided that the test is properly carried out and the organisms used are true to type and agglutinable by type sera. A positive reaction is definite evidence of past inoculation or of infection past or present by the bacillus. It is found in persons who have received prophylactic inoculations and in typhoid carriers. The former will often react 2 years after the injection and sometimes many years after the latter are commonly those who have recovered from the febrile state but continue to harbour—possibly for the remainder of their lives—bacilli in their bodies usually in the gall bladder. With these two exceptions a positive reaction is diagnostic of the disease but is rarely obtained before the end of the first or the beginning of the second week though an appreciable increase in the agglutinins is usual within the first 4 or 5 days of the onset of symptoms. The reaction usually remains positive throughout the course of the disease and for some weeks or even a few months after the temperature has become normal. A partial reaction may persist for a year and sometimes for much longer.

In the typhoid and paratyphoid infections there is usually a rise in the agglutinin titre for the group bacteria as well as for the actual infecting organism owing to the presence of the common somatic agglutinogen, but this is overshadowed by the much greater rise in the specific agglutinin for the infecting bacteria. The laboratory diagnosis of typhoid or paratyphoid fever in the case of inoculated persons is more

difficult, but can usually be arrived at by one of two methods or a combination of them both. The one method necessitates a series of tests in order to determine the fluctuations, if any, in the titre. The inoculated person who acquires a fever other than typhoid has a constant titre in his serum or the titre not uncommonly falls. Typhoid in the inoculated person nearly always produces an appreciable rise in titre. The other method depends upon the observation that the agglutinin rise after inoculation is mainly of the flagellar type, and in typhoid infections very largely of the somatic type. With a test organism treated by heat or alcohol to remove the flagellar agglutinin or a strain such as T 901, which has only the somatic agglutinin, the inoculated person's serum has little or no action unless typhoid infection supervenes.

The reaction is of less value as a basis for prognosis, a mild case of typhoid fever may never give a strongly positive agglutination test and fatal cases may or may not react strongly.

In the very early stages of typhoid fever the diagnosis should be made by the isolation of the bacilli from the blood (p 119). In the later stages, if the agglutination test is doubtful, the bacilli may be obtained from the faeces (p 387), or less frequently from the urine (p 388).

The agglutinins in diseases other than typhoid fever. Mixed infections are met with in all water borne diseases derived from faecal contamination. Typhoid, the paratyphoid and Gaertner bacilli may be present together in a contaminated article of diet the ingestion of which might lead to acute food poisoning, temporary recovery, and, after a period, typhoid fever. The serum from such a case would contain agglutinins to all the bacteria involved, and their presence could be demonstrated by carrying the serum dilutions to the extreme point at which agglutination for each organism is detected. Non specific agglutinins for allied bacteria may also be present, and a clearer recognition of the causative organisms concerned may be obtained by absorption methods which enable the specific agglutinins for each organism to be separated and estimated. Thick suspensions of the bacilli are added to equal volumes of the serum incubated for 2 hours at 37° C and centrifuged. The supernatant fluid will then be free from agglutinins for the organisms with which it has been incubated, and can be tested for agglutinins against the other members of the group.

In the case of single infections by any one member of a closely allied group such as the Salmonella group, similar absorption tests may be necessary in order to identify the infecting agent

Dysentery of the bacillary variety and such of the intestinal affections as may be caused by one or other of the dysentery bacilli give rise to specific agglutinins in the blood. The agglutination test with the serum of dysenteric patients is conducted in exactly the same manner as is the Grunbaum-Widal reaction. The amount and the activity of the agglutinin present in dysentery commonly fall short of that produced in typhoid fever and it is exceptional to meet with a serum capable in dilution of 1 in 50 of completely agglutinating one of the strains of dysentery bacilli in less than 1 hour. The dysentery bacilli differ from each other in a manner similar to the paratyphoid bacilli. the most widespread infecting agents of bacillary dysentery are those first described by Shiga in Japan and Flexner in America. Other dysentery bacilli are referred to on p 121. Agglutination reactions in suspected bacillary dysentery should be performed with both *B Flexner* and *B Shiga* and preferably with the V W X Y Z bacilli in addition

Food poisoning Gaertner's bacillus and the Aertryck bacillus are commonly met with as the causative agents in food poisoning. The diagnosis rests upon the isolation of the organism and the demonstration of agglutinins in the blood of those affected

Brucella infections The organisms producing Malta fever, tularæmia and the undulant fever caused by *Br abortus* are described on p 116. In all of these infections attempts should be made to cultivate the bacilli from the local lesion if one is present, from the blood stream or from the urine. It often happens that the diagnosis is not suspected until a late period of the fever and blood culture may be unsuccessful. Fortunately, a high agglutinin titre is usual and the agglutinin test may be the only means of making the diagnosis. There is considerable cross agglutination between the three members of this group and it is particularly important that the test organism used should also be tested against both a known anti serum and a known normal serum. A properly controlled positive reaction is definite evidence of infection by one of the group

Typhus fever The relationship of this disease to the rickettsiae and to proteus bacilli has been referred to (pp 142 and 168) The agglutination test with the proteus bacilli is known as the Weil Felix reaction and is positive in some 96 per cent of cases The agglutinins appear about the fourth day of the disease and the serum commonly reaches a high titre

Virus diseases In the majority of virus infections evidence of immunity is usually shown by complement deviation tests but in the case of small pox flocculation tests are specific and of value The antigen employed is obtained from the crusts of the patient's vesicles and the antibody from the serum of a hyper immunised rabbit

The agglutinins as evidence of infection The isolation of an organism from some part of the body is not of itself evidence that the organism is actually producing disease It may also happen that a bacterium not generally recognised as capable of producing disease or a mixture of bacteria amongst which the infective organism is in doubt may be isolated in cultures from a lesion or from the excreta or from one of the body fluids In such cases and particularly when the organism in question is a member of the colon or typhoid group evidence of the infectivity of the bacterium may be sought for by examining the agglutinating power of the patient's serum upon it together with that of the serum from a normal person The presence of agglutinins in the serum in greater than the normal amount for the organism isolated is evidence of actual infection by that organism Absence of agglutinin for the organism is no evidence against its infectivity since some bacteria give rise to little or no agglutinin in the blood even when they are undoubtedly producing disease Infection of the urinary tract with the *bacillus coli* for example commonly produces no appreciable rise in agglutinin for this bacillus

The agglutinins as a test for an organism Just as a patient with an unknown disease may be proved to be infected with a known organism by the demonstration of agglutinins in his serum for that organism so a reversal of the process the testing of an unknown organism with a known serum may be adduced as a proof of the nature of the organism In any of the typhoid or brucella group infections the nature of a bacillus isolated from the blood can be immediately tested with a known antiserum and the identification of the organism

can be more certainly and more rapidly determined than would be possible if its recognition depended upon cultural reactions

The use of agglutinating sera for the identification of bacteria is most commonly practised in typhoid and paratyphoid fevers, in bacillary dysentery and in brucella infections

Also in lobar pneumonia it is of value to know the type of pneumococcus present, both for prognosis and for treatment by the appropriate serum One method, which can be carried through in 24 hours, is to inject a saline suspension of the sputum into the peritoneal cavity of a mouse, which is usually dead on the following day The peritoneal fluid is centrifuged slowly to remove the cells, then rapidly to drive down the cocci The cocci are suspended in fresh saline and tested for agglutination against known sera of Types I, II and III

A simple and more rapid method of typing pneumococci is given on p 239

Agglutinins are thus of great value in the identification of bacteria, and it is convenient to keep in the laboratory ice chest antisera to the typhoid paratyphoid, food poisoning and brucella group bacilli as well as to the three types of pneumococci Such sera can be obtained from any reliable source, and, if care is taken to prevent contamination, will retain their agglutinating properties almost indefinitely.

The technique of agglutination tests The reaction may be carried out by a microscopic or macroscopic method

The microscopic method and the materials required for it are as follows Serum tubes, Wright's capillary tubes and rubber teat, hollow ground slides and vaseline, cover glasses, normal saline, watch glasses, a bowl or small hand basin containing carbolic acid (1 in 20), and a 24 hours-old culture of typhoid bacilli on an agar slope

The Wright's tubes and the serum tubes can readily be made from glass tubing with the aid of a blow pipe, the glass tubing should have an outside diameter of $\frac{1}{4}$ inch

To make a Wright's tube, hold the glass tubing with one hand at each end and heat it to a red heat as near to one end as it is convenient to hold it, continually rotating the glass As soon as the heated portion is freely malleable, remove it from the flame and separate the two ends evenly, without force and moderately slowly Fuse the terminal portion of the capillary part of the tube in the flame As soon as the glass is cool enough to hold repeat the process and make a series of tubes

Leave both ends of the tube sealed until required for use then nick each end with a file and break them off. The bulbous portion of each tube should be about $1\frac{1}{4}$ inches long and the capillary portion about 9 inches. The bore of the capillary part should be nearly equal throughout tapering very slightly towards the distal end. The tubes are readily made with a little practice the points requiring experience being the size of the blow pipe flame the degree to which the glass is heated and the rapidity with which the capillary tube is drawn out. The tendency is either to heat the glass insufficiently and produce a short thick tube or to draw the ends apart too rapidly and make the tube excessively long and thin.

The *serum tubes* for collecting the blood are made in a similar manner but the bulbous portion is left longer (about 2 inches) and the capillary part is made shorter and burnt through at

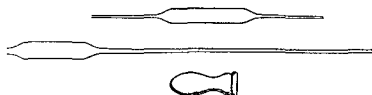


FIG 90.—Serum Tube Wright's Tube Rubber Test

its centre the distal half being left to provide the capillary portion of the next tube drawn out.

To obtain the serum cleanse the patient's thumb with ether and let it dry. If the hand is cold place it in hot water and dry thoroughly. Wind a piece of fine rubber tubing round the thumb from the base nearly to the nail. With a surgical needle make a sharp deep stab at the side of the thumb in the line of the digital artery. Avoid the pulp of the thumb. Having broken off both ends of the serum tube hold one end lightly in the drop of blood sloping the tube a very little downwards. Keep rotating the tube. When the blood has all entered the tube lay the tube gently down on a flat surface loose the rubber tubing rub the thumb briskly with a dry swab reapply the tubing and continue to fill the tube. When the bulbous portion of the tube is about half full wipe the end containing the blood warm the air in the blood free end of the bulb in the flame rapidly seal the distal capillary end pause to allow the warmed air to contract and suck the blood out of the proximal capillary end and finally seal that end also. Stand

the tube in an upright position for about half an hour or until the serum has separated then centrifuge the tube at a moderate speed. A simple alternative to this method is to take the blood from a vein with a needle and syringe (p. 224). One c.c. of blood is ample for the test.

Slides for the hanging drops should be provided with a central circular depression but this is not essential. To prepare the slides warm them in the Bunsen flame take up a little vaseline on a glass rod warm the rod in the flame to melt the vaseline and then draw the end of the rod round the circular depression in the slide leaving a substantial ring of vaseline. No vaseline should be allowed to drop into the central depression.

The *typhoid culture* used is of considerable importance. A reliable strain can be obtained from any known laboratory or the bacilli may be isolated from the blood of a case of early typhoid fever (p. 119) or from the spleen *post mortem* (p. 234). From whatever source the bacilli are derived the culture should be examined in two ways—the full cultural characters of the bacillus should be investigated (p. 118) and the bacillus should be tested against a known positive serum. The readiness with which various strains of bacilli are agglutinated is not constant and a bacillus is exceptionally met with which has all the cultural characters of the typhoid bacillus but which is not agglutinated by the most powerful sera. A reliable strain of bacilli once obtained can often be subcultured over an almost indefinite period without change of character but its agglutinability should be periodically controlled. The subculture used for performing the tests should have been incubated from the previous day on an agar slope. Before making the suspension put up a subculture on agar to preserve the strain. To make the suspension add warm saline to nearly halfway up the agar slope and replace the cotton wool plug. Gently shake the tube until the growth is washed off into the saline and the latter has become distinctly milky. The strength of the suspension used is of importance and suspensions of constant opacity should be aimed at. When a faintly milky suspension has been obtained remove the cotton wool plug and drop it in the carbolic bowl. Filter the suspension through a small filter paper moistened with warm saline and held in a pair of forceps over a watch glass. Place the culture tube and filter paper in the carbolic and sterilise the ends of the forceps.

in the Bunsen flame. The object of filtering is to remove any clumps of bacilli which may be washed off as such from the surface of the medium. The discrete bacilli pass through the filter paper.

The reaction is then performed as follows — With a blue glass pencil (or with a pen and ink) make a mark on a Wright's pipette about $\frac{1}{2}$ inch from the end. By means of the rubber teat which must fit tightly to the pipette draw up a volume of the blood serum to be tested to the mark, admit a column of air and draw up 9 equal volumes of normal (0.85 per cent) salt solution leaving a small column of air between each volume. Blow out serum and saline into a clean watch glass and mix thoroughly. the serum is now diluted to 1 in 10. Draw up a volume of the diluted serum to the mark and an equal volume of the suspension of bacilli. Mix in a watch glass and transfer a sample drop with the pipette to the centre of a cover glass spreading out the drop so as to cover about one fourth the area of the glass. Pick up the cover glass by pressing on it one of the vaseline ringed slides. Turn the slide over. Press down the cover glass with a mounted needle over the whole circumference of the vaseline ring so that no air can gain admittance. No vaseline should touch the hanging drop. Mark on the slide the time and the dilution of the serum (1 in 20). Take another volume of the diluted serum in saline and 4 volumes of the suspension of bacilli. Mix and prepare a hanging drop as before. The dilution of the serum is now 1 in 50. Take a third volume of the diluted serum and 9 volumes of the bacillary suspension and prepare a hanging drop in which the serum is diluted 1 in 100. Make a fourth drop from a sample of the typhoid suspension to which no serum has been added and label the slide control. Examine each slide with the microscope vertical and a $\frac{1}{4}$ inch objective closing the aperture of the diaphragm until the refractile bacilli are clearly seen. Observe at intervals the motility of the bacilli and the formation of clumps. Compare the drops containing serum with the control. if the bacilli in the latter lose their motility or come together in clumps the test is valueless. it is however rare to meet with a sample of typhoid bacilli which agglutinate spontaneously in suspension. In a positive reaction between serum and bacilli the latter become motionless and collected into compact masses easily visible to the naked eye few if any bacilli remaining isolated between the clumps. After com

pleting the reaction place the slides (after partially slipping off the cover glasses), the capillary tubes, typhoid suspension, watch glasses, and everything which can possibly have come in contact with the bacilli into the carbolic basin and leave them there till the next day. Make sure that no bacilli have been spilt on the bench, but if they have soak the bench in carbolic. Wash your hands after completing the reaction and do not smoke while it is being performed *

It is advisable in every suspected case of typhoid fever to repeat the reaction with paratyphoid A and B bacilli, using the same dilutions as for *B. typhosus*. If a double quantity of the original 1 in 10 dilution of serum in saline is made it will be sufficient for the three dilutions of each of the three bacilli. The paratyphoid cultures are obtained and treated in the same way as the typhoid culture. Paratyphoid B cultures are more apt to show some degree of spontaneous agglutination and are more readily agglutinated by non specific sera in strong dilution (*e.g.*, 1 in 20) than the other members of the group.

The *interpretation of the reaction* is based upon the time taken for the completion of the reaction and upon the dilution of serum capable of producing agglutination of the bacilli. With a strong serum clumping may take place within a few minutes in the 1 in 100 dilution. In a completely negative case no clumping occurs in the 1 in 20 dilution, and the motility of the bacilli may even be accelerated. Complete clumping in the 1 in 20, partial clumping with incomplete loss of motility in the 1 in 50, and little or no reaction in the 1 in 100 is a "partial" reaction. *The essentials of a positive reaction are that clumping and loss of motility should be complete in the 1 in 50 dilution within half an hour.* Such a reaction is the strongest possible evidence of typhoid infection. Complete absence of reaction in the 1 in 20 dilution is strongly opposed to the diagnosis of typhoid in a febrile case of any duration. A partial reaction is often of assistance when taken in conjunction with the physical condition of the patient, and in cases of doubt should be repeated after a few days' interval.

It may be stated here, and cannot be too strongly emphasised, that the deductions drawn from any pathological test should

* Never lay down a pipe or a cigarette on the bench. Never sit on a bench. Never leave an unattended Bunsen burner alight. Always wipe the oil from an immersion lens. These are four simple rules frequently transgressed.

never be made from the test alone. The interpretation of the results requires a knowledge of the clinical condition of the patient coupled with an understanding of how the test is performed and what it means.

The macroscopic method usually employed is that of Dreyer and permits of proportionate dilutions of the serum and bacteria. The serum dilutions are taken into small tubes in a metal stand and are made by the drop method. The size of drop of any particular fluid depends upon the external calibre of the point of the pipette used and standard dropping pipettes are supplied with the outfit which can be obtained from Messrs. Baird & Tatlock, Hatton Garden, E.C. To perform the test dilute the serum 1 in 10 by placing 2 drops in a tube and adding 18 drops of saline. Place five small tubes in a metal rack and put 10 drops of diluted serum in the first, 5 in the second, 2 in the third and 1 in the fourth. Make up the volume in each tube including the fifth (control) to 10 drops with saline. To each tube add 15 drops of suspension of killed bacilli. The pipette must be held vertical in measuring the drops and after holding serum in any dilution it must be dried by washing out with water, spirit and ether in that order. The rack is left in the water bath at 56° C. for 3 hours in the case of a negative reaction. The dilutions obtained are 1/25, 1/50, 1/125 and 1/250.

In a positive reaction clumps of bacilli visible to the naked eye and readily recognised with the help of artificial light and a dark background will form in tubes 1 to 4 and finally sink to the bottom leaving a clear supernatant fluid; the control tube will remain evenly turbid. In typhoid fever the titre commonly rises above 1/250; in dysentery it is often considerably below this figure.

The suspensions of bacilli used are preferably made with dead bacteria and it is advisable to purchase suspensions of known agglutinability and strength. Standard suspensions which will keep for 6 months can be obtained from the Department of Pathology, University of Oxford.

Zone phenomenon. In titrating the agglutinin content of a serum it may sometimes happen that in one zone of the dilutions no agglutination of the bacteria takes place. This zone is usually occupied by the tubes containing the strongest serum and is most likely to occur with heated or old sera of high potency. In all agglutinin titrations it is therefore

advisable to use a sufficient range of dilutions to exclude the possibility of error from this source

Hæmagglutinins

Red cell agglutinins are normal constituents of the serum, but, owing to their similarity to bacterial agglutinins, are conveniently considered here

If human sera and washed red cells in saline suspension from a group of different individuals are brought in contact, it will be found that the sera of some persons agglutinate the red cells of others. The red cell clumps thus found are seen as tightly packed masses under the microscope and are commonly visible with ease to the naked eye. This agglutination of red cells may progress to actual hæmolysis *in vivo* but *in vitro* lysis of the cells is rarely observed. This interaction between serum and cells is the basis of a classification of the human race into four groups. These groups in the Moss classification are as shown in the table (Fig 21) —

		MOSS			
		SERUM			
CELLS		1	2	3	4
		—	+	+	+
	2	—	—	+	+
	3	—	+	—	+
	4	—	—	—	—

FIG 21 — Blood Groups

In Jansky's classification, which is widely used on the Continent, and has the claim of priority Groups II and III are the same as in the Moss classification, but Groups I and IV are transposed

More recently it has been customary to designate the blood groups by letters instead of numbers. Group IV (Moss) becoming Group O, Group III becoming Group B, Group II Group A, and Group I AB. The letters correspond to the two cell agglutinogens A and B, the agglutinins of the serum are referred to as α and β . Thus Group AB contains both agglutinogens A and B, but no agglutinin. Group B contains agglutinogen B and agglutinin α , Group A contains agglutinogen A and agglutinin β , Group O has no agglutinogen and both agglutinins α and β .

The inheritance of blood groups follows the Mendelian law,

and has some medico legal importance. Parents of certain groups may have children of another group but A or B agglutinin never occurs in the red cells of a child unless present in one of the parents. Consequently, if both parents are Group O or IV (Moss), the children can only be of the same group. Other inherited group limitations can be easily deduced from the foregoing information an exercise which can be recommended to the student, for example if the parents are both of Group II or A, the children cannot be of Group I or AB or of Group III or B.

The existence of these blood groups has an important bearing upon the procedure of blood transfusion. Transfusion of a patient with blood, the red cells of which are agglutinated by the patient's serum, causes immediate symptoms, such as dyspnoea, precordial pain, urticaria and later hæmoglobinuria, and if sufficient blood is given death may rapidly ensue.

It is essential therefore that the cells of the donor should not be agglutinated by the serum of the recipient. Hence Group IV (Moss) donors can be used for any case in an emergency.

It is however preferable and, in cases of long standing grave anaemia essential that both donor and recipient should be of the same blood group since agglutination of the patient's cells by the donor's serum may take place after an interval and cause serious reactions.

Blood grouping. Prospective donors or patients can be grouped beforehand as follows —

Materials required. Wright's capillary tubes, slides and cover slips, citrated salt solution, a 10-c.c. syringe and sera of Groups II and III. The sera of the known groups can be obtained from any large laboratory, and it is often feasible to group sufficient of one's acquaintances to have individuals of these groups at hand. The sera can be kept on ice for many months.

Ten c.c. of blood are taken from the vein of the prospective donor's arm and a note is made of the suitability of the arm veins for transfusion purposes. About 1 c.c. of the blood is added direct to about 5 c.c. of citrated salt solution (sodium citrate, 0.85 per cent, sodium chloride, 0.85 per cent, in distilled water). The remainder of the blood is put into a test tube for the Wassermann test (p. 190), which should be done in every case.

With a Wright's tube and teat take up one volume of the Group II. serum and one volume of the citrated cells, mix, seal the tube, remove teat, and incubate for 15 minutes at 37° C. Break off the end of the tube, replace teat and expel contents on to a slide. Cover with a glass and examine under the microscope, using a $\frac{2}{3}$ inch objective. Repeat with Group III serum.

Agglutination is usually obvious to the naked eye, and doubtful readings under the microscope are unusual. A reference to the table (Moss) enables the unknown red cells to be allotted to their proper group. Thus if the cells are agglutinated by Group III serum, they must be either of Group I or Group II, if the former they will be agglutinated by Group II serum, if the latter they will not.

Once an adult donor has been grouped the allocation may be regarded as permanent. In grouping the patient a sample of the serum should always be taken as well as the red cells. Having obtained the patient's blood group from the red cell examination and sent for the appropriate group donor, any possibility of error should be excluded by cross grouping, and two preparations should be put up, one of the patient's serum with the donor's red cells, the other of the patient's cells with the donor's serum. There must be no agglutination in either preparation. In cases of emergency and when standard sera are unavailable it is permissible to proceed as follows —

Take a small quantity of blood from the patient's finger into a Widal tube and allow it to clot. Take a few drops of blood from the donor's finger into citrated salt. Mix a volume of the patient's serum with the donor's red cells in a Wright's tube. Stand at room temperature for 10 minutes and examine on a slide. If no agglutination occurs the transfusion can be proceeded with.

Blood transfusion is the most suitable procedure for replacing blood lost. It is called for after severe hæmorrhage from large vessels, but only if the bleeding points can be controlled, as a preliminary to operation likely to be attended by shock or loss of blood, in patients with severe anæmia, due to previous blood loss or sepsis, and as a temporary measure in such morbid states as pernicious or aplastic anæmia. Transfusion by blood of the correct group may be followed by untoward symptoms, such as vomiting, fever and rigors. These may usually be avoided by strict attention to details of technique.

and particularly if citrated blood is used to the following. The sodium citrate must be chemically pure and sterile the water must be freshly distilled and recently sterilised (an incredible superstition is prevalent among nurses and even medical men, which ascribes permanent sterility to water distilled into unsterilised flasks or even open vessels). It is further most important that no clotting should take place in the donor's blood whether precautions are taken to prevent these clots passing into the recipient's vein or not. This entails that the operation of transfusion should go smoothly and, simple as is the process, it frequently happens that some hitch occurs, consequently it is advised that no one, except in case of emergency, should attempt a blood transfusion unless he has watched at least once an experienced person performing it.

There are two main methods of performing transfusion. The whole blood may be taken into a vessel coated with paraffin such as a Kimpton's tube, or the blood may be citrated. The latter method is described here. The simplest apparatus to use is that of Keynes, which can be put together in any laboratory or obtained from Messrs Allen and Hanbury's Wigmore Street. It consists of a flat bottomed, narrow necked litre flask provided with a side tube. A rubber cork is fitted to the neck and pierced to allow the passage of a glass tube which reaches from the bottom of the flask, passes through the cork and is bent over to connect with an air lock, to the further end of which is fitted rubber tubing. The side tube is connected by pressure tubing with a short length of glass tubing containing cotton wool to act as a filter, and a rubber bellows is fitted to the distal end of the filter. The needle used for the donor should be, as a rule, stouter than that for the recipient, and its lumen should have a diameter not exceeding 2 mm. Needles for both donor and recipient must have points with short bevels and sharp cutting edges, and wide shoulders to fit closely metal junctions tied to the tubing. The tubing attached to the recipient's needle should be of sufficient length and fastened with an easily detachable clip just above the needle.

To take the blood proceed as follows. Remove bellows, cork and glass tubing from flask. Pour into flask 50 c.c. sterile 2 per cent sodium citrate solution (if approximately 450 c.c. of blood are to be taken). Lightly plug flask with sterile gauze

and stand it in a basin of warm water on a low table or stool

The donor—preferably lying on a couch—has adjusted round the upper arm a tourniquet or if available, an air band, which permits of adjustable pressure being made with a pump. The skin is painted with iodine the tourniquet is tightened and the vein made as hard as possible. The gauze is removed from the flask the tubing inserted (both needle and tubing having been previously sterilised) the gauze plug is laid over the neck of the flask and the needle is plunged in in upward direction through the skin into the vein. When the blood flows from the needle the tourniquet is loosened the metal junction is fitted into the needle and the flask is kept gently and constantly rotated to prevent clotting. The blood should flow readily otherwise clotting will occur and by periodically applying moderate pressure with the tourniquet 450 c.c. can be collected in about 10 minutes. The tube is then removed and the flask plugged. The blood should be used at once but can be kept for 12 hours or more on ice.

To give the blood. Insert delivery tube and rubber cork into flask. Fill rubber tubing through barrel of air lock with warm sterile normal saline excluding air bubbles and clip close to metal junction. Fix air lock barrel to glass delivery tube and note that the bottom of the tube reaches the bottom of the flask. Fix rubber bellows to side tube of flask. Gently rotate flask to mix the blood. Place tourniquet on recipient's arm and insert needle in the same way as for the donor. Remove tourniquet.

When the blood flows from the needle remove clip from delivery tube fix metal junction and pump gently. The citrated blood passes up the glass tubing flows over into the air lock the barrel of which should remain about half full. If the needle is in the vein the blood flows steadily if outside a swelling appears in the tissues round the needle. Care is taken to keep the lower end of the delivery tube always below the level of the fluid in the flask and a constant watch is kept on the air lock. After insertion of the needle in either the donor or recipient's vein the shoulder of the needle is held in position with the fingers throughout the operation.

In addition to the normal interaction between the sera and cells of different individuals two pathological phenomena in which agglutination or hæmolysis occurs in the individual may be referred to here.

Autoagglutination is a rare occurrence resulting in the agglutination of the patient's red cells in his own plasma at the moment the blood is shed, and is *not connected with any specific morbid state*. In a well marked example the condition is readily detected. On pricking the lobe of the ear almost a clear plasma exudes, having suspended in it agglutinated masses of red cells. A blood count in such a case is quite impossible.

Paroxysmal hæmoglobinuria, following exposure to cold, and frequently associated with syphilitic infection, has a specific hæmolysin in the serum which can be demonstrated in the following way. Blood is taken from the vein and immediately clotting has taken place the serum is separated and placed on ice for 5 minutes or longer. On subsequent removal it is mixed with the washed red cells either from the patient or a normal individual and placed in the incubator at 37° C. Hæmolysis then occurs. The citrated blood of the patient may be placed directly on ice, and subsequently incubated, when marked hæmolysis occurs but if the whole blood is thus used the stay on ice must not exceed 5 minutes.

OPSONINS

The nature of opsonins. Opsonins were shown by Wright to be substances present in the blood which have the property of acting upon bacteria in such a manner that the phagocytes are able to ingest them. Opsonins are present in considerable amount in health, they may be either increased or diminished as the result of infection. Opsonins are for the most part destroyed by heating the serum to 60° C and also by keeping it, they thus differ from the agglutinins, and closely resemble the complement of normal serum.

The opsonic content of a serum is estimated by incubating together a suspension of bacteria, washed normal leucocytes and the serum to be tested. After incubation stained films are made of the mixture, and a given number of phagocytes are counted together with the number of bacteria ingested by them. If this is repeated with a normal instead of the patient's serum the opsonic index can be estimated. The index is rarely used now either as a guide to treatment or as a means of diagnosis, and there is fairly wide agreement that actual

variations in the index in disease are, on the whole, less than the variations essential to the technique of the test

ANTIGEN—ANTIBODY—COMPLEMENT REACTIONS

There are a number of immunity reactions which, in diagnosis are known as complement deviation tests. They depend upon the specific interaction of antigen with antibody, and the subsequent linking up of the non specific complement. The adsorption or linking up of complement is demonstrated by the addition of sensitised red cells.

Complement is the non specific thermolabile substance present in all fresh sera. It can be destroyed by heating the serum to 56°C , or adsorbed upon any particulate substance added to the serum.

Antigens are substances capable of producing antibodies, thus toxins give rise to antitoxins, bacteria to bacteriolysins, red cells to hæmolysins. To act as an antigen a substance must have chemical complexity, and it is probable that all antigens contain protein molecules. Further, antigens must be given parenterally, that is, by some other route than the alimentary tract. Immunisation to some substances given by the mouth is practised, but can only be successful if the antigen so given is absorbed unchanged into the circulation.

Sensitised red cells are red cells united to their specific antibody in the absence of complement. They are obtained by immunising an animal of one species to the red cells of an animal of a different species. The immunised animal is bled, the serum is heated to 56°C to destroy the complement and then added to a suspension of the washed red cells in saline of the other animal. Such red cells become hæmolysed on the addition of complement and can be used as an indicator for the presence of free complement in any antigen antibody complement mixture. If no hæmolysis occurs both antigen and specific antibody must have been present, since both must unite before complement is absorbed. If hæmolysis does occur, then either antigen or antibody is absent.

The complement deviation reaction can be applied as a method of diagnosis in various affections. Bordet and Gengou found, for example, that if the serum of a patient convalescent from typhoid fever were heated (to destroy the complement, but not the antibody) and incubated with typhoid bacilli

(antigen) and the equivalent amount of guinea pig's serum (complement) and then added to sensitised red cells no hæmolysis took place because typhoid bacilli, typhoid antibody and complement had united and no complement remained for the sensitised red cells whereas if normal human serum (heated) were incubated with typhoid bacilli and guinea pig's serum and then added to the red cells hæmolysis took place readily since normal serum contains no antibody to the typhoid bacillus and has therefore no substance capable of uniting with the bacilli and the complement in the guinea pig's serum. Consequently complement is available to combine with the sensitised red cells and hæmolyse them. This reaction is known as the Bordet Gengou reaction or the complement deviation test. It is a specific test. Antibody can unite only with its specific antigen. Specific antigen and specific antibody must both be present before any combination with the (non specific) complement can take place.

The Wassermann reaction is an application of the Bordet Gengou test to the diagnosis of syphilis. In the case of syphilis the specific antigen is the *Spirochæta pallida* and since this organism had not been cultivated at that time Wassermann conceived the idea of utilising some organic extract rich in spirochætes and for this purpose made use of an extract of the liver of a syphilitic fœtus. The reaction was completely successful since it was found that the heated serum of a syphilitic patient incubated with guinea pig's complement and the extract of syphilitic liver absorbed by virtue of the syphilitic antibody the complement from the mixture whereas normal serum failed to do so. The test appeared to afford a specific proof of infection by the spirochæte.

The meaning of the test is so far clear. It has however since been demonstrated that the Wassermann reaction is not an essential combination between syphilitic antigen and antibody since the spirochæte can be altogether omitted from the mixture. If an alcoholic extract of human heart muscle or even of guinea pig heart muscle be substituted for the syphilitic liver extract the reaction is equally reliable inasmuch as it is obtained with syphilitic and not with normal sera. The essential substances in the extract employed as antigen are found to be certain fatty bodies or lipoids such as can be extracted from normal organs. Why lipoids are capable of acting as syphilitic antigen in place of the true antigen the

spirochæte is unexplained. It has been suggested that the syphilitic virus has an affinity for the body lipoids and combines with them forming a toxo lipid and that an antibody is produced to the toxo lipid which would be capable of combining with it or with lipid. According to this explanation the reaction is a combination between anti toxo lipid present in syphilitic serum lipid acting as antigen in place of the toxo lipid and complement.

The reaction may be viewed from the physical rather than the immunity standpoint and some knowledge of both conceptions is essential since the reaction as a test for syphilis may be based on the reasoning of immunity as in the Wassermann method or upon the physical change which takes place in the serum as in any of the precipitation tests. Syphilitic sera have acquired the property of precipitating lecithin and it is probable that this property results from some alteration in the globulin fraction of the serum proteins. The precipitate formed on adding syphilitic sera to cholesterolised alcoholic tissue extracts is visible to the naked eye and forms the basis of the flocculation test methods. If any precipitate forms in a serum containing complement the complement will be adsorbed so that the basis of the original Wassermann test may be the precipitation of lipoids by the altered globulin the adsorption of complement upon the precipitate and the subsequent failure to hæmolyse red cells.

Whatever may be the explanation of the reaction it follows that since the spirochæte may be replaced as antigen by lipid a positive reaction is not evidence of a specific immunity to *S. pallida* but rather of a peculiar and abnormal lipid metabolism such as might be common to more than one disease. A positive Wassermann reaction has not therefore the specific character of a true immunity reaction and is evidence rather of actual infection by the spirochæte than immunity to it.

The reaction in diagnosis. Since the reaction is not a truly specific one we might expect it to be present in some diseases other than syphilis and this is found to be the case. A positive reaction is associated with certain diseases foreign to this country including sleeping sickness yaws and some forms of leprosy. A positive reaction is also found occasionally and for a brief period only in scarlet fever and in glandular fever. With these exceptions—and from the point of view of diagnosis

they are unimportant exceptions—a positive Wassermann reaction is very definite evidence of a syphilitic affection. As to the occurrence of the reaction in the various stages of syphilis —

In *primary syphilis* the reaction becomes positive in from 2 to 6 weeks after infection. A negative reaction at any date later than this in the case of a doubtful sore is strongly opposed to the diagnosis of syphilis. Since it is of the utmost importance however to commence treatment at the earliest possible date and whenever possible before the reaction has become positive there is fortunately no necessity to rely upon the Wassermann test. In cases of any doubt the presence of the specific spirochete is conclusive (p. 229).

In *secondary syphilis* if untreated the test is practically always positive and a negative reaction almost excludes syphilis.

In *tertiary syphilis* positive reactions are not quite so invariable: the test is however positive in from 80 to 90 per cent of treated and untreated cases. It is more common to find a positive reaction in some tertiary lesions than in others: a negative reaction with a thoracic aneurysm for example is extremely rare.

In *latent syphilis* that is to say in cases of past infection with no present manifestations of the disease a positive reaction is evidence of liability to recurrence of the disease. A reaction which remains consistently negative for at least two years rarely if ever reverts to positive. Such a negative reaction is not however certain evidence of cure but is still consistent with syphilis particularly of the central nervous system.

In *neuro syphilis* the serum reaction is almost invariably positive in general paralysis in about 90 per cent of cases of meningo vascular syphilis but in only from 60 to 70 per cent of tabetics. Further information is obtained by examination of the cerebro spinal fluid (p. 272). The Wassermann reaction is rarely positive in the spinal fluid in secondary syphilis a lymphocytosis with increased protein content and a negative reaction being more common. In meningo vascular syphilis the reaction is positive in about half the cases: in tabes it is positive at some stage of the disease but may be negative during the remissions. In general paralysis a positive reaction is almost invariable. In all forms of neurosyphilis a lymphocytosis with an increased protein content is usual in the spinal

fluid, the increased protein being accompanied by a relative and absolute increase in the globulin

In *congenital syphilis* the reaction is practically always strongly positive at birth, but tends to become weaker as age advances

The Wassermann test is thus seen to be of great value in the diagnosis of all syphilitic lesions, but it must be remembered that a negative reaction sometimes occurs with lesions undoubtedly syphilitic and that a positive reaction means that a patient is tainted with syphilis—it does not necessarily mean that the particular lesion for which he is under observation is syphilitic. In the case of a doubtful tumour, for example a negative reaction is evidence against syphilis, a positive reaction yields the valuable information that the patient has had syphilis and is probably still infected, but it does not tell us that the "tumour" is a gumma

The response of the reaction to treatment The alterations which may take place in the reaction as the result of treatment depend partly upon the efficiency of the treatment and partly upon the stage of the disease at which treatment is being undertaken. In the secondary stage of the disease the reaction alters rapidly in response to energetic treatment, and should become negative in from 8 to 12 weeks. During the treatment the reaction may be seen to change from strongly positive to partial and finally to negative. A negative reaction at this stage does not mean that the patient is cured unless it remains negative for many months, and possibly not even then, since if further treatment is omitted a negative reaction may again become positive and be followed by a relapse in the symptoms and physical signs. If the treatment is inadequate during the primary and secondary changes the reaction remains positive or becomes partial only, and, since there can be little question that a permanently negative reaction is the goal to be aimed at, the effect of treatment is reasonably controlled by the state of the reaction, which is a more sensitive guide than the clinical condition of the patient. In the tertiary stage of syphilis the behaviour of the reaction is quite different, since at this stage, whatever the treatment adopted, it is exceptional to obtain a completely negative reaction, and frequently very little change is observable in it. The behaviour of the reaction in this stage of syphilis is comparable with the effect of treatment in the clinical condition of

the patient, since it is notoriously difficult to cure the disease after tertiary symptoms have manifested themselves. In the tertiary stage lesions frequently clear up rapidly under treatment, but have the greatest tendency to recur, and comparatively few cases are actually cured.

The technique of the Wassermann reaction There are numerous methods of performing the reaction and they differ considerably from one another. There is, however, a general consensus of opinion that the test employed should correspond in its main features with the original Wassermann reaction, and that if a flocculation test or any other serious deviation from the original technique is adopted, this should be regarded as an *adjuvant* and not a *substitute*. Two methods are given here, the first being a modification of the original reaction and the second a flocculation test.

Method 1 The Wassermann reaction The materials required are large and small test tubes, standard graduated pipettes of 10 c.c. and 1 c.c., normal saline, two water baths at 56° C. and 37° C. respectively, metal racks to hold the small tubes and in addition, fresh guinea pig's serum (complement), immune serum or amboceptor (rabbit to sheep) washed red cells (sheep) antigen, human sera (normal, syphilitic, unknown).

The *complement* is fresh guinea pig's serum. It is obtained by plunging a needle attached to a 5 c.c. syringe into the animal's heart, withdrawing the blood and ejecting it into a centrifuge tube. The guinea pig is usually none the worse for the operation.

The blood is allowed to stand at room temperature overnight in order to allow the titre of the complement to settle. It is then centrifuged and the clear serum pipetted off.

The *immune serum* is the serum of an animal which has been inoculated with washed sheep's corpuscles. The sheep's blood is obtained from the butcher and received into sterile citrated salt solution. It is then centrifuged and washed several times with saline. A 50 per cent suspension of the cells is made in saline and three doses each of 1 c.c. are injected into the vein of a rabbit at one hourly intervals. One week later 3 c.c. are given. The rabbit is bled 7 to 10 days later, but it is somewhat a matter of chance when a serum acquires a high titre. The rabbit's blood is removed by a needle and syringe from a vein in the ear, and after standing is centrifuged. The

clear serum is pipetted off into sterile capsules. The capsules are sealed and heated in water at 60° C for 15 minutes. They are then stored and the sera will keep their specific attributes for several months.

The preparation of the immune sera involves unnecessary labour for most clinical laboratories and a reliable amboceptor can be obtained from Burroughs Wellcome & Co.

The *red cells* are those of a sheep and are obtained and treated in the same manner as those used for injection into a rabbit. A 5 per cent saline suspension of the final washed deposit is used for the reaction.

The above materials—namely the complement, the amboceptor of the heated immune serum and the red cells—constitute the hæmolytic system.

The *antigen* is prepared as follows. A heart is obtained from any cadaver in the *post mortem* room and slices of the muscular portions are removed with a clean knife, blotted dry and weighed. The slices are transferred to a mortar and thoroughly ground up with absolute alcohol in the proportion of 1 gram of heart muscle to 9 c.c. of alcohol. The mixture is transferred to a well fitting glass stoppered bottle and shaken occasionally for 1½ hours. It is then filtered into a similar bottle labelled

heart extract and kept in the cold. Into another bottle weigh 1 gram of pure cholesterol, add 100 c.c. of absolute alcohol. Shake and heat in water bath until the cholesterol is dissolved. Label and store in the cold. Extracts obtained in this way are extremely constant in strength and will keep without variation for some months.

The *human sera* are best obtained by venæ puncture and preferably by the aid of a syringe (see blood culture p. 224). The syringe must be dry to avoid hæmolysis and from 5 to 10 c.c. of blood are taken and ejected into a dry test tube. After standing to allow the clot to contract the serum is pipetted off into centrifuge tubes, centrifuged and the corpuscle free serum stored in small labelled test tubes in the cold until required for the test. No serum should be kept longer than 7 days.

The *salt solution* is made in the usual manner from pure sodium chloride and distilled water. For the purposes of this reaction it should be freshly prepared and of a strength of exactly 0.85 per cent.

Standardisation of the materials. The most variable reagent

is the immune serum and this is standardised as follows. A 1 in 1 000 dilution of the serum is made with saline, and into a series of tubes 0.1, 0.2 up to 0.9 c.c. of this diluted serum are put and each tube is made up to 0.9 c.c. with saline. To each tube is added 0.1 c.c. of a 1 in 2 dilution of guinea pig's serum and 0.5 c.c. of the 5 per cent suspension of sheep's red cells. The tubes are incubated for 1 hour at 37° C. and then allowed to stand aside for the corpuscles to settle. The minimal hæmolytic dose is the dilution of amboceptor in the first tube showing complete hæmolysis and an amboceptor should be rejected if the dose does not fall between these dilutions, namely between 0.0001 and 0.0009 c.c. Five times the minimal dose is used in the test. The complement used in the standardisation should be the mixed sera of two or more guinea pigs.

The standardisation of the amboceptor need not be repeated for several months and if the serum used is one purchased from a reliable firm the hæmolytic titre which accompanies it can be accepted. It is not essential to standardise the antigen if it is carefully made but it is mixed and diluted with saline just before use in the following proportions: heart extract 0.8 c.c., cholesterol 0.53 c.c., saline 18.6 c.c. giving 20 c.c. or enough for forty tubes. The heart extract and cholesterol are first measured with pipettes into a bottle after drying out the pipettes and bottle with absolute alcohol. The saline is then poured in a steady stream on to the mixture which then becomes strongly turbid. The complement is standardised in the preliminary stage of each series of tests.

To perform the reaction —

Stage I. The sensitised red cells.

Measure 2 c.c. of the washed and centrifuged deposit of red cells into a stoppered bottle. Measure 35 c.c. of saline into a graduated cylinder. Add with a 1 c.c. pipette the requisite volume of amboceptor (if the titre of the amboceptor is 1 in 1 000 five times the m.h.d. for 40 c.c. of red cell suspension is $\frac{40 \times 5}{1\,000} = 0.2$ c.c.) and make up the saline to 38 c.c.

Add the saline amboceptor mixture to the red cells and stand in the incubator at 37° C. until required.

Stage II. Titration of complement.

Into seven large test tubes in a rack put 0.1 c.c. of complement in tubes I to IV.

To tube I add 0.9 c.c. saline, dilution = 1 in 10
 „ II „ 1.9 c.c. „ „ = 1 in 20
 „ III „ 2.9 c.c. „ „ = 1 in 30
 „ IV „ 3.9 c.c. „ „ = 1 in 40
 „ V „ 1 c.c. of tube III + 1 c.c. of saline = 1 in 60
 „ VI „ 1 c.c. of tube IV + 1 c.c. of saline = 1 in 80
 „ VII „ 1 c.c. of tube IV + 2 c.c. of saline = 1 in 120

Into seven Wassermann tubes put 0.5 c.c. of each of the above dilutions and add 1 c.c. of saline and 0.5 c.c. of sensitised red cells to each tube. Place in water bath at 37° C for 30 minutes. The minimal hæmolytic dose is the dilution of the last tube showing complete hæmolysis, and three times this dose is used. Thus, if tube V is the tube containing the m.h.d., the complement is diluted twenty times.

Stage III The sera are diluted and inactivated as follows: the Wassermann tubes are placed in three rows in a metal stand, and three tubes are required for each serum. Put 0.1 c.c. of serum in each tube and add 0.9 c.c. of saline to tube I (back row) 0.2 c.c. to tube II and 0.4 c.c. to tube III (front row).

Put rack in water bath at 56° C for 30 minutes.

Spinal fluids are thus diluted. Five tubes are used for each fluid. 0.1 c.c. 0.2 c.c. up to 0.5 c.c. of spinal fluid is measured into the tubes and the content of each tube made up to 0.5 c.c. with saline. It is advisable to heat the diluted spinal fluids in the same manner as the sera.

Stage IV Add 0.5 c.c. of antigen to the middle and front rows of serum tubes and to all the spinal fluid tubes. Add 0.5 c.c. of complement to all the serum tubes and an extra 0.2 c.c. to the middle row (the total content of each serum tube is now 1.5 c.c.). Each spinal fluid tube receives 0.5 c.c. of complement. Place all tubes in water bath at 37° C for 45 minutes.

Stage V Add 0.5 c.c. of sensitised red cells to all tubes and replace in water bath at 37° C until the back row of the serum tubes shows complete hæmolysis. This should take about 15 minutes.

Reading of results The results may be read immediately after the conclusion of Stage V, but it is advisable to stand the tubes in the ice chest overnight and compare the results the next morning. If a back row serum tube shows no hæmolysis, the serum is anti-complementary and the test is

useless. Such a result may occur if the serum has been stored too long or is deeply hæmoglobin tinged, and the test should be repeated with a fresh specimen. Sera properly taken are very rarely anti-complementary. Complete hæmolysis in all three serum tubes is a completely negative reaction (— —). No hæmolysis in middle and front row tubes is a strongly positive reaction (+ +). Numerous intermediate reactions occur, and partial hæmolysis in any tube may be designated \pm . The spinal fluid tubes are usually returned in the same manner with results from 5 + marks to 5 — marks. A definite + in tube 5 only is a feebly positive reaction.

Method 2 The Kahn precipitation test is a modification of the Sachs Georgi method, and the technique given below is that employed at the Venereal Clinic at the London Hospital.

The antigen is a cholesterolised alcoholic extract of powdered beef heart which has had part of the ether-soluble matter removed. Since antigens prepared from different samples of heart muscle vary in sensitivity, it is advisable to use many hearts in the production of an antigen. Unless a large amount of antigen is to be made up this is wasteful and can be avoided by the use of a commercial product known as "Bacto Beef Heart" manufactured by the Digestive Ferments Company, Detroit, U.S.A. and supplied in this country by Baird and Tatlock. This product is powdered, dried beef heart and is produced on a large scale by mincing up hundreds of beef hearts stripped of the fat, the minced heart muscle is then dried by electric fans and subsequently ground into a fine powder.

Preparation of the antigen (1) To 25 grams of the Bacto Beef Heart powder in 250 c.c. Erlenmeyer flask add 100 c.c. of ether. Shake well (at intervals) for 10 minutes. Filter, decanting as much of the ether as possible, press any powder on the filter paper with a spatula until the ethereal liquid is expressed and then return the powder to the flask.

(2) Extract the powder in the flask in a similar manner with 75 c.c. of ether three times and dry the residual powder in the air until no odour of ether can be detected.

(3) The powder is then weighed and returned to the flask. For each gram of the powder add 5 c.c. of 95 per cent alcohol. Shake the flask well for 10 minutes and allow the alcohol extraction to proceed at room temperature for 3 days.

(4) At the end of this time shake the flask well for 5 minutes.

and filter the extract off. Measure the quantity of extract obtained and then place in a clean ground glass stoppered bottle and for each cubic centimetre of alcoholic extract add 6 mgm of cholesterol (Kahlbaum).

Then place the bottle in the 37.5° C incubator overnight, and it will be found that the cholesterol has dissolved and the antigen is then ready for titration.

Preparation of special pipettes * When a large number of sera have to be tested much time and apparatus is saved if two pipettes have been made so that the proportions of antigen and serum may be maintained. Thus in the test the serum:antigen ratio of the two tubes is 6:1 (0.15 c.c. to 0.025 c.c.) and 12:1 (0.15 c.c. to 0.0125 c.c.). These ratios may be obtained if a fine capillary dropping pipette be made for measuring the antigen by drops. 24 drops from which equal 1 volume delivered from a graduated Wright's pipette which is used for measuring the serum. Thus if 1 volume of serum (= 24 drops) be added to 4 drops of the antigen a ratio of 6:1 is obtained. In like manner 1 volume (= 24 drops) added to 2 drops of the antigen gives a ratio of 12:1.

These pipettes are made as follows —

(1) *Antigen dropping pipette* Take a piece of glass tubing ($\frac{1}{8}$ inch diameter) and draw out to a fine capillary point. Cut squarely at the narrowest diameter. Fit rubber teat to the wide end.

(2) *Serum delivery pipette* Take an ordinary Wright's pipette fitted with a rubber teat and rinse out with alcohol. With the antigen dropping pipette carefully drop into the bottom of a Wassermann tube 24 drops of a mixture of equal parts of absolute alcohol and saline (0.85 per cent). Draw the mixture up into the Wright's pipette and make a mark at the upper end of the liquid column when its lower end coincides with the tip of the pipette. By repeating this manoeuvre a few times the mark can be checked or adjusted until the pipette delivers a volume † as nearly as possible equal to 24 drops from the antigen dropping pipette.

Titration Standardisation of the Antigen This is necessary to determine the *minimum* quantity of salt solution giving

* This method of measuring the reagents used in the reaction has been tested in parallel with Kahn's standard technique and found to give identical results.

† The volume is quite arbitrary but about 0.2 c.c. is a suitable amount.

aggregates with a constant volume of antigen which are completely dispersed upon the further addition of salt solution

Technique (1) Measure 0.3 cc 0.4 cc 0.5 cc 0.5 cc and 0.6 cc of salt solution (0.85 per cent) into five Kahn tubes

(2) Measure into five Kahn tubes 0.5 cc of the antigen

(3) Prepare five antigen suspensions by mixing the 0.5 cc quantities of antigen with the varying amounts of salt solution in series. This should be done by emptying as rapidly as possible the saline into the antigen and then pouring the mixture back and forth five or six times

(4) Allow the mixtures to stand for 30 minutes

(5) Set up two rows of five Kahn tubes one behind the other in a Wassermann rack. Thus there are five sets consisting of two tubes—one set for each antigen saline suspension

(6) Using the antigen dropper pipette and beginning with the antigen suspension containing the most saline drop in series 2 drops of each antigen suspension into the front row tubes and 4 drops into the back row tubes respectively

(7) Using the serum delivery pipette measure into each tube 1 volume (— 24 drops of the antigen dropper pipette) of saline

(8) Shake the rack for 3 minutes in a shaking apparatus at a speed of 275 to 285 oscillations per minute

(9) Examine the tubes using a hand lens ($\times 6$) and a slit lamp for the presence or absence of a fine granular precipitate

(10) That pair of tubes containing the suspension in which no precipitate is seen and the fluid is clear but opalescent is taken as the titre. This is usually 1:1

The test proper The test employs two reagents the antigen suspension and the sera to be tested. A requisite amount of antigen suspension depending on the number of sera to be tested should be made up according to its titre. Thus if the titre is 1:1 equal amounts of saline and antigen should be mixed by pouring the former on to the latter as rapidly as possible and then pouring the mixture back and forth five or six times. The mixture is allowed to stand for 10 minutes before use. All sera to be tested should be inactivated at 56° C. for 30 minutes

Two rows of Kahn tubes are set up in a Wassermann rack one behind the other each serum to be tested having one tube in the back row and one tube in the front row

To each tube in the back row 4 drops of the antigen suspension are added by means of the antigen dropping pipette. To each tube in the front row 2 drops of the antigen suspension are added by means of the antigen dropping pipette. One volume of each serum to be tested is added to its appropriate tubes in the back and front rows respectively by means of the serum delivery pipette. It should be noted that after each serum has been added the serum delivery pipette should be carefully rinsed out with saline (0.9 per cent). The rack containing the tubes should now be shaken on the mechanical shaker (275 to 285 oscillations per minute) for 3 minutes. This may be done by hand approximating the speed of the mechanical shaker as nearly as possible but the results apart from the process being very fatiguing are not so reliable.

Reading of results This can be done by one of three methods —

- (1) Direct observation looking into the tubes
- (2) Using a concave mirror and examining the image in the mirror
- (3) Using a slit lamp and making observations with a +6 lens

Of these three methods the slit lamp is to be preferred, since conditions of observation are constant.

Recording results *Positive* Definite easily visible particles floating in a clear or opalescent fluid. The particles may vary in size depending on the strength of the reaction but are quite obvious.

Doubtful positive Extremely fine particles floating in a somewhat turbid medium.

Negative The fluid is transparent opalescent and quite free from particles.

A comparison of the two methods The Kahn test demands more labour in the preparation of the antigen and it is essential that the dilutions of the stock antigens used in the test should not precipitate spontaneously. The actual performance of the test is shorter and apparently simpler than that of the Wassermann reaction. The simplicity of the Kahn test, however, is more apparent than real. Very exact measurements of antigens and sera are essential and the reading of the results requires considerable experience. In skilled hands the Kahn and Wassermann tests correspond in about 85 per cent of cases, the discrepancies being partly accounted for by the higher

sensitiveness of the Kahn test which remains positive longer than the Wassermann test. The employment of the Kahn test with spinal fluids is not advised.

The flocculation method should be used as an addition to the Wassermann test and not as a substitute for it. The performance of the Kahn test by those untrained in serological technique as the sole test for syphilitic infection is indefensible.

The complement fixation test in other diseases. Before the test described above was applied to the clinical diagnosis of syphilis it had been demonstrated in numerous other infections by Bordet and Gengou. The test is applicable in cholera typhoid whooping cough and other diseases but is not constant during the infection and is only strongly positive during convalescence. The test is considerably used in the diagnosis of gonorrhoeal infection. The antigen employed is derived from a mixture of several strains of gonococci which are grown on solid media and washed off in saline. The saline suspension is heated to 60° C for 1 hour and the number of organisms estimated as in the preparation of vaccines (p 204). A suitable strength for the antigen is 4 000 million cocci per cubic centimetre. With all bacterial antigens it is advisable to use in the test not more than half the amount which fixes the complement of a normal serum. In human tuberculosis no satisfactory diagnostic test of this nature has yet been published though several methods are still under consideration. The difficulty lies in the preparation of a satisfactory antigen.

In hydatid disease the test may be used as a clinical method of diagnosis. The antigen is here the fluid from a hydatid cyst and if this be substituted for the lipid extract the test for the presence of hydatid infection may be carried out in the same manner as the Wassermann test for syphilitic infection. Different hydatid cyst fluids vary greatly in their antigenic content. The cyst fluid must not be filtered or heated and no antiseptics can be added. It is necessary therefore to obtain fluid in a sterile manner from an uninfected cyst and to standardise it. On theoretical grounds it might be expected that the complement fixation test would be applicable in all diseases in which the specific infecting body or antigen is known. In practice the test as a routine clinical method is at present almost entirely confined with the exception of hydatid infections to the diagnosis of syphilis. It is however

employed in the diagnosis of many virus infections, including chicken pox, small pox, zoster and psittacosis. In these conditions the margin between the specific and non specific absorption of complement in the test is much smaller than in the Wassermann reaction, though sufficient for diagnosis in practised hands.

Complement deviation tests may also be used for the identification of an infecting organism just as agglutination tests may be.

VACCINES

A vaccine is a sterile standardised suspension of dead organisms in a neutral fluid. It is given hypodermically in doses graduated according to the number of bacteria present in each dose. The object of vaccine treatment is to raise the resistance of the individual to the organism with which he is infected by carefully graded and spaced doses of dead organisms. The toxins contained in the dead bacteria are liberated in the tissues, passed into the circulation and thus lead to a production of antibodies which can be used to combat the living organisms of the disease. Less frequently vaccines are given to a healthy individual with the view of rendering him immune to a possible infection. The immunity induced by vaccines is an artificial acquired immunity. It is established comparatively slowly, over a period of weeks, and lasts a comparatively long time.

The treatment of a patient with vaccines necessitates a co-operation between the pathologist and the clinician. The cure of a case of gonorrhœal arthritis, for example, may be greatly expedited by means of a vaccine, but the vaccine may be almost useless in the presence of an untreated stricture. In such a condition as infective endocarditis, in which the source of infection cannot be dealt with, vaccine treatment is useless.

There is no doubt that vaccines have been given in the past in a wild and extravagant manner, and the field of vaccine therapy is now becoming more restricted. The following are among the conditions for which vaccines may reasonably be given —

Chronic local infections, such as recurrent boils and local abscesses, of which the causative organism is certainly known, and is usually a staphylococcus.

Acute streptococcal infections, particularly those in which

the organisms have been isolated from the general circulation and the local source of infection is accessible

Gonorrhœal infections particularly when chronic less certainly in the stage of acute urethritis

Chronic catarrhal conditions of the respiratory tract are frequently benefited by vaccines. There is little reasonable doubt that the common cold is the result of a virus infection but that the continued purulent discharges and other complications are due to secondary infection by the ordinary pyogenic bacteria of which the most common are streptococci pneumococci and staphylococci. Immunity to the virus infection appears to be very short lived but it is possible to raise the resistance to the secondary invaders and a course of vaccine treatment in the early autumn is justifiable using vaccines prepared from the pyogenic bacteria and particularly the hemolytic streptococci present in the throat of an individual who suffers each winter from recurrent and long-continued colds.

In the following conditions the use of vaccines is more problematical but worthy of trial in individual cases —

Pyorrhœa alveolaris and its probable complications

Chronic infection of the urinary tract with the *B. coli*

Local abscess such as a bronchiectatic cavity or a secondarily infected tubercular lesion in which the organism isolated is the probable source of the secondary infection

Vaccines should not be given merely for the sake of doing something. A coccus extracted from the healthy gums or normal faeces of a hopeless rheumatic subject and made into a vaccine is a futile and dishonest procedure.

Stock vaccines should not be given on the chance of hitting off the correct species of organism when it is possible to make a proper bacteriological investigation and to grow the causative organism.

While it is probable that the majority of vaccines do no harm it must be recognised that in some cases the injection of dead organisms is positively dangerous. An overdose of vaccine given during the height of an acute infection may turn the scale against the patient. In a chronic staphylococcal infection the giving of vaccines for too long a period may produce a state of hyper-susceptibility to the infecting organism and each injection may be followed by a fresh abscess. In such cases and in any examples of wide spread

and chronic infection, treatment by toxoid is preferable to that by vaccines. Staphylococcal toxoid is prepared by growing the organism on soft agar in a partial atmosphere of CO_2 , grinding up the agar and filtering through a Seitz filter. The filtrate, or toxin, should have a hæmolytic action of high titre for rabbit red cells. Approximately 0.3 per cent of formalin is added and the mixture incubated for 2 to 3 days or until all hæmolytic power has gone. The first dose should not exceed $\frac{1}{2}$ c.c. of a 1 in 50 dilution.

The above are only the merest indications of when or when not to treat a patient with vaccines. Each case must be judged on its merits but it can be stated broadly that the cases most benefited are some chronic septic conditions and general streptococcal infections. The dosage of a vaccine necessarily varies with the age, size and general condition of the patient as well as with the species of the organism injected. The variations as to age and condition are similar to those employed in any form of treatment. The dosage according to species of organism, varies with the comparative virulence of the organism. In the cases of streptococci, pneumococci, and gonococci small initial doses of from 5 to 10 million cocci are given as a general rule. In the cases of staphylococci and colon bacilli doses of from 50 to 100 million organisms are given. Double these amounts may usually be injected in the second dose.

The vaccine may be given as soon as it is prepared, but if a surgical operation has recently been performed, and there is reason to suppose that the patient has absorbed a material dose of toxin from the operation area, the giving of the initial dose should be postponed. If there is urgency in giving the vaccine, a probable idea of the causative organism justifies the use of a preliminary dose of a stock culture of that organism. Nor is there any grave objection to treatment by stock vaccines if the proper organism is known, although the balance of evidence seems to be that patients are more likely to improve on an autogenous vaccine—that is, a vaccine prepared with the organisms obtained from the individual case—than on a vaccine derived from some other source. The time allowed to elapse between the doses is as a rule from 5 to 10 days. There is nothing to be gained by increasing the size of the dose to much more than double the initial dose, nor by markedly diminishing the intervals between the doses.

In most cases the patient experiences little or no discomfort after the vaccine is given, but sometimes a definite reaction follows. The reaction may be local, focal or general or all three. The local reaction consists of pain, redness and even œdema at the site of injection. The general reaction comprises a rise of temperature with headache, malaise and sometimes sickness. A focal reaction consists in an aggravation of the local condition. A severe reaction means an overdose for the individual.

Certain modifications of vaccine treatment may be mentioned only since they have yet to be put into practice on any large scale. Treatment by endotoxins has been attempted for several diseases, and in particular for typhoid fever. The endotoxins are obtained by grinding up the bacilli in such a way as to actually express from them the intracellular toxins. The sensitised vaccine of Beredka consists of a vaccine which has been brought into contact with the specific antiserum so that the organisms in the vaccine are combined with the antibody in the serum. It is claimed that such a vaccine is non-toxic, leads to no preliminary lowering of the immunity, raises the immunity very rapidly, and produces an immunity which lasts a considerable time. The so-called detoxicated vaccines are suspensions of bacteria, the toxic elements of which have been removed and the antigenic properties left. Evidence of such separation and of the therapeutic value of these preparations is indecisive.

Tuberculin is given in many different forms and only one variety, tuberculin B E, is a genuine vaccine, in the sense that it consists of a known weight of tubercle bacilli emulsified with a mixture of glycerine and water. The old tuberculin consists of exotoxins mainly, and is obtained by evaporating down a 4 to 5 weeks' old culture of bacilli to one tenth its bulk at a comparatively low temperature, filtering it and using the filtrate. Tuberculin T R is composed of endotoxins and 1 c.c. of the T R contains the bacterial matter insoluble in water derived from 10 mgm. of tubercle bacilli which have been repeatedly washed in water and centrifuged. The S B E consists of sensitised tubercle bacilli obtained by mixing the bacilli with anti-tuberculous serum.

The preparation of the tuberculins is largely left to commercial firms.

The dosage of the tuberculins varies with the nature of the

case and is reckoned so far as possible from the weight of original solid substance employed to make the extract or from the original solution

In the case of B E for example the preliminary dose is a dilution of the original solution and is commonly 0.001 of a cubic millimetre. This may be given as the initial dose of any tuberculin. The dose is increased gradually and in such a way as the following — 0.001 0.002 0.003 0.004 0.006 0.008 0.010 0.015 0.02 0.03 etc. The maximum dose to be aimed at is 100 c. mm. or $\frac{1}{10}$ c. c. of the original solution.

The intervals between the doses should be 3 days for the small doses and from 1 to 3 weeks when the higher doses are reached.

The rate of increase necessarily depends upon the reaction and progress of the patient.

The value of tuberculin treatment cannot even yet be considered as proved and there is no doubt that in careless hands much harm can be done. In general it may be said that all reactions local general or focal are to be avoided that skin glandular joint and genito urinary tuberculosis may be expected to benefit from tuberculin treatment in experienced hands. The treatment of pulmonary tuberculosis is disappointing.

The method of preparing vaccines. The following are the various stages requisite for the preparation of an autogenous vaccine —

Stage 1. The culture. Cultures are taken from the patient in the usual way and film preparations of the lesions are also examined if possible. A sub culture on agar (or if necessary for the growth of the organism on serum agar) is made and incubated for 24 hours. A second sub culture should be put up at the same time for the purpose of full cultural investigation. If the organism is in pure culture there is no difficulty at this stage and the original culture on agar may be used if there is any urgency about the preparation of the vaccine. If more than one organism is present it is advisable to make the vaccine from the more virulent bacterium particularly if it predominates in the film preparation and is most likely from the nature of the case to be the primary cause of the lesion. For example if a *Staphylococcus albus* and a *Streptococcus pyogenes* are obtained from an acute cellulitis the vaccine should be made from the streptococcus. In cases of

doubt or when there is reason to suppose that a combination of organisms is responsible for the condition, vaccines can be prepared from two or more organisms and subsequently mixed in whatever proportions are considered desirable

Stage 2 *The suspension* To the 24 hours' old sub-culture on an agar slope add sterile 1 per cent salt solution. The amount of saline to be added depends upon the thickness of the growth. A moderately turbid suspension of bacteria in saline is to be aimed at. Wash off the growth as far as possible into the saline by shaking the tube and pipette off the bacterial suspension into a small sterile tube, the mouth of which can be drawn out and sealed in the flame.

Stage 3 *The standardisation* The number of bacteria per cubic centimetre of a suspension can be gauged with sufficient accuracy by comparing the opacity in a standard tube with a set of known standards. Such standards can be obtained from Messrs Burroughs Wellcome & Co.

Stage 4 *The sterilisation* Prepare a deep water bath or a saucepan capable of holding a considerable bulk of water. Fill almost to the brim with water at 60° C. Place over a Bunsen flame and lower the flame considerably. Leave a thermometer in the water. By adjusting the size of the flame the temperature can be maintained at 60° C without appreciable variation. When the temperature has become constant immerse the tube in the water. Keep the tube at 60° C for 1 hour observing the reading of the thermometer from time to time. At the end of 1 hour remove the tube. The actual temperature necessary to kill the organism varies with the species. The majority of organisms growing in pin point colonies such as gonococci streptococci and pneumococci are killed by exposure to 60° C in from 30 to 45 minutes. Organisms growing in coarser colonies such as staphylococci and colon bacilli require a temperature of at least 65° C for an hour and with some strains it may be necessary to raise the temperature higher still.

Stage 5 *The proof of sterilisation* Incubate the vaccine tube for 24 hours. At the end of this time sub-culture from the vaccine on to an agar slope and incubate the sub-culture for a further 24 hours.

If the sub-culture remains sterile, the vaccine may be considered sterile.

Vaccines killed by heat lose their antigenic properties on

keeping and should not be used after more than 3 months have elapsed. Stages 4 and 5 may be substituted by killing the bacteria with chemical agents. Carbolic acid is added to the suspension in the proportion of $\frac{1}{2}$ c.c. to 100 c.c. and the mixture is put aside for 2 to 3 days or until a sub culture in broth remains sterile. It may be necessary with some bacteria to raise the strength of carbolic from 0.5 to 0.75 per cent.

Stage 6 *The dilution* Calculate the convenient dilution of the bacterial suspension. For example if a streptococcal suspension has been found to contain 500 million cocci per cubic centimetre and the initial dose wished for is 10 million it is convenient to add 4 volumes of diluent to 1 volume of the suspension thus reducing the strength of the suspension to 100 million cocci per cubic centimetre. The initial dose will then be contained in one tenth of a cubic centimetre.

Sufficient carbolic should be added to the water used for dilution to provide in the final mixture a carbolic solution of a strength of 0.5 per cent. The addition of the carbolic safeguards the vaccine from subsequent contamination.

The mixture is finally placed in a sterile bottle and kept in a dark cool place. The bottle should be marked with the name of the patient, the nature of the organism, the strength and the date.

The above steps are those preferably observed. It will be noticed however that 2 days are required for the sub culture and 2 more for the proof of sterilisation so that if everything goes well 4 days are required for the preparation of the vaccine.

In cases of urgency a stock vaccine may be given at once.

In acute streptococcal infections it is possible to prepare a vaccine in 24 hours. The original culture on agar is taken and if pure the suspension is made and heated to 65°C . for 1 hour and for 10 minutes at 70°C . It is then presumed sterile and is diluted and injected. There is in practice no real risk in this procedure.

To give the vaccine A 1 c.c. hypodermic glass syringe graduated in one tenths of a cubic centimetre is required. The syringe is conveniently sterilised with boiling methylated spirit. A small quantity of the spirit is placed in a beaker and brought to the boil on a sand bath over a Bunsen burner. The flame is turned out when the spirit is boiling briskly and the syringe with needle attached is washed in and out several times with

the boiling spirit. The vaccine bottle must be thoroughly shaken before drawing up into the empty syringe the required volume of the vaccine. The injection is preferably made subcutaneously into the forearm after cleansing the skin with iodine.

The prophylactic use of vaccines. Vaccines may be given with the object of preventing disease. Almost the only preventive vaccination of this kind commonly performed in this country is that for typhoid fever. Vaccination against small pox is of a somewhat different nature since the individual is protected by an artificial attack of the modified disease. The effects of inoculation against typhoid are to lessen markedly the risk of infection and to modify the virulence of the attack if it does occur. There is sufficient statistical evidence that a considerably smaller percentage of inoculated persons become attacked after exposure to infection than of untreated persons and that the incidence mortality rate among the inoculated is very low. The effect of the inoculation gradually wears off and the comparative immunity does not appear to last longer than from 1 to 2 years. Typhoid fever is not sufficiently prevalent in Britain to call for preventive inoculation but inoculation should be advised for those proceeding to countries where the disease is rife. While abroad all the usual precautions against infection should be observed and re inoculation should be performed if practicable within 18 months. The vaccine should be given a short time before the typhoid district is reached. Two doses are given with a 10-day interval between them. Comparatively large doses are commonly given often from 500 to 800 million organisms and it is preferable to give a combined vaccine prepared from typhoid and paratyphoid bacilli. The inoculations in a considerable percentage of cases are followed by a definite local reaction and often by slight pyrexia and malaise.

The Pasteurian method of inoculating animals against anthrax and human beings against rabies consists in giving a series of injections of the infecting agent of increasing virulence. Haffkine's anti-cholera vaccine is given on a similar principle. The anti plague vaccine of Haffkine consists of a killed broth culture of plague bacilli grown for 6 weeks at 25° C.

Calmette has been conducting on a large scale a prophylactic method of inoculation against tuberculosis. His vaccine is prepared from a strain of tubercle bacilli of bovine origin.

which has been cultivated for many years in ox bile. The strain, known as the bacillus of Calmette and Guérin or BCG, is acid fast and is claimed to have lost its pathogenicity for man and animals, but to have retained its antigenic properties. Large doses of living BCG have been given hypodermically or by the mouth in an attempt to produce immunity in susceptible persons. Calmette and his followers have carried out prophylactic inoculation on a very large scale, and claim both freedom from any ill effect and success in prophylaxis. These claims have however been subjected to considerable criticism.

The diagnostic use of bacterial products. The reaction of the skin to bacterial toxins is made use of in the diagnosis of disease and in the estimation of immunity. In tuberculosis an infected person is more susceptible to the action of tuberculin than a normal individual and this hypersensitiveness is made use of as a diagnostic procedure. The test most frequently employed is that of Von Pirquet in which the skin is scarified through a drop of 35 per cent. old tuberculin diluted with glycerin. Ten minutes later the tuberculin is wiped off and in a positive reaction the skin becomes reddened and a papule forms in about 24 hours. A more delicate method in children is that of Mantoux in which 0.1 c.c. of 1 in 1000 old tuberculin is injected intracutaneously as follows. An area of the skin is cleansed with ether and the needle attached to the syringe, is worked upwards into but not through the skin, the needle being held almost flush with the forearm. When the point of the needle is in position in the skin it is visible as a dark line, and pressure is then put upon the piston of the syringe. The drop of fluid, as it is forced out, makes a white bubble in the skin, like a nettle sting, and is evidence of the injection being intradermal. A control injection of normal saline is made on the opposite forearm. The reaction is observed in 24 hours, and, preferably, again in 48 hours. With a positive reaction an angry red area of the size of a five shilling piece appears around the site of inoculation, and often the lymphatic vessels are injected and the lymph glands enlarged. Not infrequently there is a rise of temperature.

The ophthalmic reaction of Calmette, in which the tuberculin is dropped into the conjunctival sac, is apt to produce a severe and intractable conjunctivitis in sensitive persons and has been largely abandoned.

The tuberculin reaction is only reliable within certain limits. Patients dying of tuberculosis may not react at all and patient with healed tuberculous lesions may react strongly. Owing to the large number of adults with quiescent tuberculous foci a positive reaction in an adult is little evidence of active tuberculosis. A negative reaction in an adult is evidence against tuberculosis. In children the reaction whether negative or positive is of considerable value.

Other diagnostic reactions are occasionally performed on similar lines. A cutaneous and an ophthalmic reaction have been made use of as a test for typhoid fever and the subcutaneous injection of mallein is largely practised in veterinary pathology as a means of detecting glandered horses.

In the diagnosis of *lydatid* disease a complement deviation test may be used (p 198) or a cuti reaction performed with the fluid. A small portion of the sterile cyst fluid is injected by the intradermal route. In *diphtheria* and more recently in *scarlet fever* the intradermal injection of the specific bacterial toxin has been made use of as a measure of the immunity to infection.

The *Schick reaction* in *diphtheria* is performed by injecting into the skin a minute amount of diphtheria toxin usually $\frac{1}{4}$ mld and comparing the result on the following day and again on the third or fourth day with the control injection of heated toxin made in the opposite arm. Those who react are called *Schick positive* and having no neutralising properties in the blood to the toxin are susceptible to infection. Those who do not react—the *Schick negative* individuals—are immune to *diphtheria* and though they may be carriers of the bacilli will not get infected by them. By this means any community can be divided into immunes and susceptibles. Further the *Schick positive* persons can then be immunised by giving subcutaneously at intervals of 5 to 10 days two or more injections of diphtheria toxoid obtained by treating the toxin with formaldehyde. Their subsequent reactions will be found to have become negative. The *Schick method* is of great value in large institutions and in the presence of epidemics. It is not infallible but exceptions are rarely met with. The toxin for the diagnostic test and the toxoid for immunisation can be obtained from a few of the leading firms and the doses prescribed by them can be accepted.

The *Dick test* in *scarlet fever* in many respects resembles the

Schick test in diphtheria, and appears to depend upon the ability of the individual to neutralise a measured quantity of streptococcal toxin. The toxin, which, unlike diphtheria toxin, is thermostable, is usually obtained by growing hæmolytic streptococci from a scarlatinal source in rabbit blood broth for 5 days and filtering. The filtrate, a toxin, is injected as in the Schick test and the dose is usually 0.1 of a 1 in 1,000 dilution. The positive reaction appears in 6 to 12 hours. The reaction is negative in convalescents, but positive in the first few days of scarlet fever.

Protein sensitivity tests. A group of morbid conditions presenting phenomena resembling those met with in the anaphylactic state are, in some cases associated with a sensitivity to certain proteins. Among these conditions are asthma, hay fever, and urticaria. For diagnostic purposes the skin of the arm is lightly scarified with a surgical needle over an area the size of a threepenny piece, and the protein extract is gently rubbed in. The number of possible proteins to which the patient may be sensitive is very large, and, if no clue is derived from the patient it is advisable to test half a dozen or more of the "group" proteins *eg*, milk egg fish, meat, bacteria, pollen, etc. If a reaction is obtained the different members of the group may be tested out. A dozen or more areas can be inoculated on one arm and a careful chart kept of the corresponding proteins. A positive reaction is usually evident in about 20 minutes but it is advisable to make a second inspection the following day. Hay fever patients frequently show a reaction to one of the pollens, but a definite result in asthma is the exception. If an article of food is incriminated it can be excluded from the diet, or if this is not possible, and if a pollen or bacterial extract is the cause, desensitisation may be attempted by a course of vaccine treatment. The various protein extracts required are preferably obtained from a reliable firm.

ANTI-SERA

Anti sera consist of the blood sera of animals—in the majority of cases horses—which have been highly immunised against bacteria or their toxins. The injection of such sera into human beings is the method of artificially producing a passive immunity. The immunity thus conferred is very

different from that aimed at in vaccine therapy since the antibodies are in the case of sera manufactured by the animal and injected into the human body. The resulting immunity is in consequence rapidly produced and of short duration.

The antitoxic sera are in most instances standardised on the basis of the amount of toxin which they can neutralise. In the case of diphtheria antitoxin the smallest amount of diphtheria toxin capable of killing a guinea pig of 250 grams weight within 4 days is first determined. This is known as the minimum lethal dose. The amount of antitoxic serum which will neutralise 100 times the minimum lethal dose is found by mixing serum and anti serum and then injecting the mixture into a guinea pig without causing death. This amount is reckoned as 1 unit of antitoxin.

The anti sera are obtained by the repeated inoculation of horses with bacteria or their toxins until a high degree of immunity is attained in the horse's serum. The animal is then bled from the jugular vein into a sterile receptacle. The clear serum which results after standing or centrifuging is pipetted off and stored in sterile glass capsules in measured doses. The anti sera are given to human beings by subcutaneous injection into the loose tissues of the axilla or abdominal wall or intramuscularly. In cases of urgency the intravenous route may be employed, but is by no means devoid of risk. The dose may be repeated in 24 or 48 hours but it is rarely advisable to give more than 3 doses and dangerous to continue for more than a week.

In favourable cases the temperature drops to normal within a few hours and the local and general condition rapidly improves.

The injection of serum is not infrequently followed by the appearance of a diffuse and irritating rash usually of the nature of urticaria and less frequently by painful effusions into the joints.

The severe anaphylactic phenomena consisting of rapid collapse and death which are readily induced in animals by repeating the injection of the serum after an interval of 2 to 3 weeks are very rarely observed in man. Serum reactions are to be expected in a patient who has previously received serum treatment and the sensitivity of such a person may be tested by his skin reaction as described in the paragraph on protein sensitivity. If a marked dermal reaction takes place the serum should be injected in the small initial dose of 0.05 c.c.

and the amount doubled every half hour until the requisite number of units have been given

The preparation of anti sera is beyond the scope of ordinary clinical pathology since the use of large animals is involved and reliable sera can be obtained from some of the leading chemical firms

Only a brief indication of the mode of preparation and of the dosage and use of a selection of the various sera can be given here

Anti diphtheritic serum The horse is injected with the toxin of the diphtheria bacillus obtained from the filtrate of a 3 to 6 weeks old culture of the organisms in broth The dose of serum given varies from 2 to 10 000 units The longer the lapse of time from the onset of infection the higher is the dose given There can be no doubt as to the value of this serum which should be given in all cases of diphtheria

Anti tetanic serum is obtained by the injection of a horse with tetanus toxin in increasing doses The serum should be given in large doses until a total of 50 to 100 000 units have been administered The injection can be intra muscular intravenous or intra thecal The first route is preferable but the serum is not so rapidly absorbed the second is more rapid but more dangerous the third has in all probability no advantage over the other routes is more distressing to the patient and produces a considerable meningeal reaction

Anti streptococcal serum The horse is immunised by a series of injections of a single strain or a combination of varieties of streptococci of increasing virulence The present day anti sera made against the toxin of hæmolytic streptococci have considerable value in combating the toxic symptoms of these infections They should be given at once in doses of 20 c c or more and may be followed by injections of the appropriate vaccine

Anti-pneumococcus serum is prepared by immunising horses first with dead cultures and subsequently if necessary with living bacteria The sera of highest titre have been those against Type I pneumococcus and by Felton's method the serum has been concentrated and freed from 90 per cent of the serum protein More recently a polyvalent serum to Types I and II has been prepared by the same method of concentration The serum should be given intravenously and in large doses at the earliest possible stage of the disease

Anti staphylococcus sera Horses are immunised against the cocci and their diffusible toxins. The antitoxic sera are of value in acute cases with positive blood cultures.

Anti meningococcus sera may be obtained for each of the four types of meningococci or a polyvalent serum may be employed. The anti sera so far produced have varied greatly in potency, and no reliable method of titration has yet been arrived at. The serum is given into the spinal canal in doses up to 20 c c for an adult. Owing to the absence of complement from the spinal fluid the addition of fresh human serum is advised in order to render lysis effective.

Anti-colon bacillus serum is obtained by the immunisation of horses to mixed strains of colon bacilli. In acute colic infections of the kidney the effect of the serum is sometimes striking but it must be remembered that the clinical fluctuations in this condition apart from serum treatment are often remarkable. The serum may be given in 20 c c doses on 3 consecutive days.

Anti plague serum The horse is immunised with saline suspensions of the bacilli. The first doses are sterilised by heat. In the later doses living bacilli are given. The serum is usually given in 20 c c doses.

Anti-anthrax serum The serum commonly employed is that of Selazo and is derived from asses immunised by injections first of anti serum and attenuated bacilli, and subsequently of virulent bacilli.

Anti measles serum is derived from the blood of convalescents and has been used as a means of treatment or, preferably, as a method of preventing or modifying the disease in contacts. The pooled serum from convalescent cases may be employed or if this is not available, the serum of any adult who has had the disease in childhood. Infection can be prevented in contacts up to 5 days after exposure. The disease can be modified if the serum is given between 5 and 8 days after contact. The serum is ineffective after the ninth day. Prevention does not protect against a subsequent attack. Modification produces a solid immunity at the cost of a very mild attack which scarcely inconveniences the patient. The serum is given subcutaneously, and the dose should be 10 c c for children over 5 years and 5 c c for those under 5 years. If the serum is obtained from adults the dose should be doubled.

CHAPTER X

BACTERIOLOGICAL METHODS

The general apparatus required for the majority of bacteriological examinations consists of—

- Slides and cover glasses
- Platinum wire and Pasteur pipettes
- Forceps
- Culture tubes and crates
- Incubator
- Centrifuge
- Staining reagents

The slides and cover glasses require no special preparation, but are conveniently kept in methylated spirit in wide mouthed glass jars until required for use when they are wiped dry with a clean cloth. After use the slides can be placed in lysol for an indefinite time and subsequently cleaned and used again.

The platinum wire should be a moderately stout piece about 4 inches long. The wire should be fixed into one end of a glass rod about 9 inches in length by heating the end of the rod to red heat in the blow pipe flame and plunging the platinum wire into it while hot. The free end of the platinum wire should then be bent round upon itself to form a small oval loop. The returning end of the wire should exactly meet the straight wire and should not overlap it. An untidy platinum wire is often responsible for untidy and consequently both inaccurate and dangerous bacteriological work.

If comparatively large amounts of fluid are required to be transferred from one tube to another it is convenient to have either Pasteur pipettes or sterile graduated pipettes. The former are readily made from glass tubing one end of which is drawn out in a capillary length of about 6 inches, the other end being left of the calibre of the tubing and of a length of about 2 inches. The capillary end is sealed in the flame and the other end plugged with wool. The pipette is then sterilised

and when required for use the tip is broken off the capillary portion is flamed and a teat is fitted over the plugged mouth of the pipette. Ordinary graduated pipettes may be sterilised wrapped in paper or stacked in metal cases. After pipettes should be placed in lysol and those of the type are subsequently destroyed.

The forceps should be of the ordinary straight variety in dissection or operation work. It is convenient to have a pair of catch forceps of the pattern known as C. The culture tubes can be bought ready for use but a large amount of bacteriological work required is considerable it is more satisfactory and economical to prepare the media in the laboratory. The methods of making the media will be described subsequently. The essential media consist of broth and agar slopes and these are sufficient for taking the primary cultures in most cases. For the complete investigation of organisms other media are necessary. After inoculation the tubes are preferably placed in basket wire crates lined at the bottom with cotton wool. The crates can be dispensed with and ordinary glass beakers or tins may be made use of instead.

The incubator should be one capable of maintaining a constant temperature of 37°C . Reliable incubators provided with self regulating capsules are supplied by several firms. A less expensive apparatus consists of a copper chamber fitted with a water jacket and warmed by a gas jet the heat within the chamber being controlled by the size of the jet. Such an apparatus is liable to vary with any considerable fluctuations in the room temperature.

When occasional bacteriological investigations only are required a wide mouthed Thermos flask can be readily adapted. The variety of flask made to hold soups or stews is the most suitable and can be fitted with a home made copper wire framework capable of holding 4 culture tubes. The flask is filled about two thirds full with water at 40°C a temperature which in 18 hours will have dropped to about 35°C . Such an incubator is sufficiently reliable for the cultivation of diphtheria bacilli or indeed the majority of readily growing pathogenic organisms.

subcutaneous
over 5 weeks
is obtained from
centrifugal machine must be capable of starting slowly and stopping gradually. Hand-driven is useful for bringing down urinary deposits but not the best for most bacteriological work. The best

form of centrifuge is one in which the carriers are held in a circular disc or plate, which can run free when the power is cut off. With the water driven turbine type a water pressure of 40 to 50 lbs to the square inch is necessary with single-jet machines and 25 to 35 lbs with a double jet. When electricity is used as the driving force it is conveniently applied by a motor working directly on the spindle and regulated by a series of stops connected with the starting lever. A maximum speed of 5 000 revolutions to the minute is sufficient for most purposes. The noise and vibration set up by most centrifuges has been reduced to a minimum in the modern forms which are slung by chains from a stand or wall bracket. With all forms of centrifuge the tubes and carriers must be accurately balanced before being spun.

The staining reagents requisite for all ordinary bacteriological work are few in number. The majority of them can be bought ready made up in solution, and the mode of preparation of each is given subsequently.

The general procedure to be followed when investigating the bacterial content of pus or of any body fluid is as follows —

- (1) Make films of the pus

{	(a) with some simple dye such as carbol
(2) Stain them	thionin
{	(b) by Gram's method
- (3) Examine the films
- (4) Put up cultures in both liquid and solid media
- (5) Incubate at 37° C for from 12 to 24 hours
- (6) Examine the culture tubes with the naked eye and with a hand glass
- (7) Make films from the cultures. Stain and examine them
- (8) If the organism is in pure culture, sub-culture it from the solid medium into the appropriate media. If a variety of organisms is present, plate out from the liquid culture into Petri dishes
- (9) After incubation of the sub-cultures for 24 hours (or longer if necessary) examine them, note the changes which have occurred in them, and make film preparations from them

(1) To make films from pus. Clean a slide. Sterilise the platinum wire by heating it to a red heat in a Bunsen flame. *Let the wire cool in the air.* Take up a loop of the pus and spread it evenly by a circular motion so as to make a thin

round film about the size of a shilling in the centre of the slide. Sterilise the platinum wire. Dry the film by waving it in the air above the flame. Fix by passing it three times rapidly through the flame.

(2) To stain the films (a) With a simple stain. This should always be done as a routine. The examination of films made from the original fluid yields information which may be altogether missed in the investigation of the culture tubes. Also an indication is obtained of the nature of the organism, and consequently of any special stain that may be necessary, as well as of the appropriate media upon which to make the cultures.

The best general stain is carbol thionin. The advantages of this stain are that it brings out the majority of organisms and the cells pre-ent, and that it is almost impossible either to under- or over-stain the films.

To stain with carbol-thionin. Cover the entire slide with the stain. do not merely place a few drops of the stain on the film itself.

Leave for approximately 3 minutes.

Wash in tap water. Blot dry. Mount in Canada balsam.

(The mounting of any film which is to be examined with a $\frac{1}{2}$ inch objective is unnecessary unless a permanent specimen is required since the cedar oil placed directly on the film clears it.)

In place of carbol thionin, dilute methylene blue may be used. the staining process is identical.

(b) By Gram's method. A modification of the original method is as follows —

Filter methyl violet (p. 254) on to the slide.

Stain 1 minute.

Wash off stain with iodine solution (p. 254).

Cover with the iodine for half a minute.

Wash in methylated spirit and continue to wash until the violet colour ceases to run from the film.

Blot dry.

Counterstain with carbol fuchsin diluted 1 in 10 with tap water.

Stain for 2 minutes.

Wash in tap water. Blot dry. Mount.

The principle of the stain is as follows. Methyl violet stains the great majority of all organisms. If the film is

then washed in spirit the stain comes out again. But if the film, after staining, is treated with Gram's iodine the methyl violet is fixed in some organisms so as to resist subsequent decoloration by spirit, but not in other organisms. The counter stain with carbol fuchsin is not part of Gram's method, but is used to display those organisms which have lost their colour in the spirit. "Gram positive" organisms are consequently coloured violet and "Gram negative" organisms red. The distinction between these colours is more obvious by daylight than by artificial light. No fixed time is given for the decoloration stage in spirit, because the time occupied depends largely upon the thickness of the film. It must be recognised that it is possible to wash the colour out of a "Gram positive" organism or to leave the colour in a "Gram negative" one if the washing is too long or too short. The alcohol process is finished directly the violet colour ceases to run from the film, and this point is readily determined if a small quantity of clean methylated spirit is reserved for the final dipping of the slide.

In addition to a simple stain such as carbol thionin or methylene blue and Gram's method the only other staining process in constant laboratory use is that for the acid fast bacilli and is known as the Ziehl Neelsen method.

The Ziehl-Neelsen method. Filter carbol fuchsin (full strength) into a large bore test tube. Bring it carefully to the boiling point, constantly shaking the tube and keeping its mouth pointed away from the face. Cover the slides with the boiling stain. Stain for 7 minutes, and during that time pour off the stain twice and add fresh boiling stain.

After 7 minutes pour off the stain, dip the slide in water and then place in 25 per cent sulphuric acid. (It is advisable when dealing with two slides to leave one staining in the fuchsin while the other is being decolorised in the acid.)

When the slide is decolorised place it in tap water. The red colour will probably return, in which case replace in the acid and then return to the water. Repeat the process until the pink colour fails to return after immersion in water. Leave in fresh water for 2 minutes.

Counter stain in 1 per cent methylene blue, diluted four times with tap water, for 2 minutes. Wash in tap water. Blot dry.

In the preliminary staining with fuchsin all organisms and

cells are stained red. After the acid everything is decolorised except the tubercle bacillus. The final washing in water brings a brighter red colour into the tubercle bacillus. The counter stain renders other organisms and the cells blue.

(3) To examine the films. Use a No. 2 eye piece, a $\frac{1}{2}$ inch objective and, when available, daylight.

Look for the kind of cell present. In the great majority of such films these will be polymorphonuclears with an occasional mono nuclear cell and possibly a few epithelial cells.

Examine for the presence of bacteria and note whether they are cocci or bacilli, whether they are arranged in pairs, clumps or chains and whether they are mainly within the cells or outside them. If more than one variety of organism is evidently present observe which variety appears to predominate. If no bacteria are seen in the films the causative organism may still be recovered in the cultures.

(4) To put up cultures. If the pus is taken directly from the body it is commonly necessary to make both films and cultures at the same time. In such cases the culture media suitable to the suspected organism are chosen. If the pus is received in a sterile receptacle in the laboratory it is preferable to examine the films before putting up the cultures. In the majority of cases two tubes should be inoculated, and these should be a broth tube and an agar slope.

It is often advisable to add a litmus milk tube and some blood-containing medium such as a serum agar slope, since some organisms, for example, pneumococci and streptococci may grow poorly on agar in primary culture while others may not grow at all.

To inoculate the tubes. Hold the test tube containing the pus and the culture tubes between the thumb and first finger of the left hand. The tubes should not be held upright, but in a slanting direction, since dust and organisms may fall into them.

Sterilise the platinum loop in the flame. *Allow it to cool.* Place the glass handle between the first and second fingers of the left hand.

Take a pair of forceps in the right hand and with a screwing motion twist out the cotton wool plugs from the tubes. Place each plug between the third and little fingers of the left hand.

Put down the forceps on the bench.

Take the platinum wire in the right hand and pass it into the

tube of pus Take out the wire, pass it into the agar slope tube and spread it gently over the surface of the medium Dip the wire again into the pus, then into the broth tube, and shake off the pus into the broth Be careful not to touch the sides of any of the tubes with the wire Sterilise the wire and lay it down

Pick up the forceps Take each wool plug separately and alight it in the flame Put the plug still alight, into the tube Do not blow the plug out it will cease to burn as soon as it is pressed well home in the tube With a blue glass pencil mark each tube with the date and a distinguishing number

(5) *To incubate the tubes* Place the tubes in a wire crate, taking care not to knock them on the edge of the crate, and place in the incubator at 37°C till the following day From 12 to 24 hours is sufficient time in which to obtain a naked-eye growth of the majority of organisms but the cultures should not be considered sterile before the lapse of at least 4 to 5 days Exceptional organisms such as the tubercle bacilli show little or no growth before the tenth day

(6) *To examine the culture tubes* Remove the tubes from the incubator and look at them to see if any growth has taken place Note the character of the growth in broth and the size, shape, colour, and density of the colonies on the agar slope Examine the agar slope further with a hand lens

(7) *To make films from the cultures* In the case of a liquid medium hold the tube in the left hand, sterilise the platinum wire, pass it into the left hand, and allow it to cool Remove the wool plug with forceps and with the wire take up a considerable loop of the broth (or milk) culture Pass the wire back into the left hand Ignite the plug and replace it in the tube Make as thick a film as possible exactly in the centre of a clean slide Sterilise the wire

In the case of a solid medium first place a small drop of tap water on the centre of the slide, then proceeding as above, take up in the wire a small particle of the growth and rub it in the water, making as thin a film as possible Allow the films to dry Fix them in the flame Stain by Gram's method

(8) *To make sub-cultures* It is advisable in the majority of cases to "plate out" from the broth culture, but this may be dispensed with if an examination of the original films and of the cultures reveals only one variety of organism Particular

attention is to be paid to the nature of the colonies on the agar slope and if these appear identical with each other the culture may be presumed pure

When the original cultures appear to be pure sub-culture from the agar slope into those media which give characteristic reactions with the suspected organism. In most cases it is advisable to sub-culture into litmus milk neutral red broth a selection of the litmus carbohydrate broths and on to gelatin slopes

To make the sub-culture hold the agar tube and two or three of the tubes to be inoculated in the left hand. Sterilise the wire. Allow it to cool. Scrape off a colony or portion of a colony from the agar slope. Shake off the growth in a liquid medium or rub it over the surface of a solid one. Sterilise the wire. Replace the plugs. Repeat with the remaining media. Label each sub-culture with the name of the medium (particularly in the case of the carbohydrate media) the date and the name or number of the case

It is however preferable if the time permits to transfer a single colony from the original culture to a fresh agar slope incubate until the next day and then to inoculate various media from the growth derived from one colony

When the original cultures are mixed it is necessary to separate the different organisms and this is done by means of plate cultures in Petri dishes. A minimum of two plates should be used and if the organisms are varieties of cocci both plates should contain agar. If organisms of the colon group are suspected prepare one agar plate and one plate containing MacConkey's neutral red medium. The plate cultures are prepared as follows —Place stab culture tubes in a tall beaker filled with hot water to above the level of the medium in the tubes. Bring the water to the boiling point over a Bunsen burner. When the media are completely liquid turn out the gas and leave for 5 or 10 minutes. Have ready sterile Petri dishes with well fitting lids. Take a melted stab culture tube from the beaker. Twist out the wool plug with forceps. Pass the mouth of the test tube through the flame (to sterilise the outside of the glass). Lift up the lid of the Petri dish just enough to insert the mouth of the test tube. Pour the contents of the test tube into the dish. Replace the lid and rotate the dish so as to spread the medium evenly over its bottom. As soon as the medium is set insert with sterile forceps into

the upper lid a piece of sterile blotting paper cut to shape and of such a size that the overlapping edges are just caught between the rims of the upper and lower lids. The paper absorbs the moisture and prevents a film of water forming over the surface of the medium and so mixing the colonies. The dishes are then set aside until they are quite cold. To inoculate them —Take the incubated broth culture tube in the left hand. Sterilise the platinum wire. Take one loop of the broth culture. Put back the broth tube in the crate after replacing the plug. Lift up the lid of the agar plate the minimum distance. Rub the platinum wire repeatedly across the agar medium in a succession of parallel streaks. Replace the lid of the dish. Lift the lid of the second agar or the MacConkey plate and repeat the process without recharging the platinum loop. Do not breathe on the plate while filling it or while inoculating it. After from 12 to 24 hours incubation of the plate cultures examine them with the naked eye and with the hand glass. Observe the types of colony present in those parts of the plates in which the colonies are discrete. Note the colour, shape and size of the colonies. Make film preparations if necessary. Sub culture from each variety of colony on to agar slopes. Incubate the sub cultures and the following day pass them through the various media.

(9) To examine the sub cultures. Note the naked eye changes which have occurred in each tube at the end of 24 hours. Examine film preparations from one or two of the tubes. The complete changes will probably not have taken place in the media after only 1 day's incubation but with many organisms a diagnosis can be made at this stage. Indole formation in the broth tube may require at least 3 days incubation and so may the production of a green fluorescence in neutral red broth. The complete coagulation of milk, the liquefaction of gelatin and the production of an acid reaction in the carbohydrate media may require some days. It is *advisable therefore to postpone the indole test as long as possible* and to incubate all the tubes for from 5 to 7 days before discarding them.

Pure cultures In routine bacteriological work it is assumed that cultures derived from a single isolated colony are pure cultures but it is evident that a colony is not necessarily derived from a single organism and it is occasionally possible by replating the sub culture of a single colony to obtain a

mixture of bacterial species. Even an apparently single species can, by special methods, be separated into organisms growing in different types of colony. The dysentery and typhoid bacilli for example can be separated into rough and "smooth" colonies. The "rough" forms tend to grow in clumps in broth, they give unstable suspensions in saline and they differ antigenically from the smooth forms of the same species. Further, the "rough" forms obtained from a virulent strain are usually devoid of virulence. It is considered that the two types of colonies consist of different aggregations of the same species which have developed certain inherent characters at the expense of others.

Single colony cultures are still accepted as pure but the student should recognise that the assumption is not from choice but from necessity, and has led to much confusion in the investigations of such problems as the variability and mutability of species in culture. There is, unfortunately no simple method of isolating single bacteria. Single-celled cultures can be made but the technique of all known methods is difficult. The ingenious procedure devised by Barnard may be indicated here. A thin suspension of the bacteria is made in gelatin, a drop is placed on a slide, covered with a quartz slip and allowed to set. Under the microscope with dark ground illumination a field is found containing a single organism and with a needle a tiny bead of mercury is worked into position over the bacterium. The preparation is then removed from the microscope and exposed to ultra violet light. All the bacteria are destroyed, except the single organism lying beneath the mercury globule. The slide is then incubated at room temperature, and the colony which develops from the single cell is taken for investigation.

Blood cultures. In health the blood is sterile and the recovery of organisms from it during life is of pathological significance. Bacteria may invade the blood stream in demonstrable numbers under the following circumstances —

(1) *By spread from a local septic focus* as in puerperal septicæmia from the uterus or in any other streptococcal infections which commence as a local lesion. Staphylococci are most commonly grown from the blood in acute bone disease and blood cultures taken before the bone is opened are usually positive. Anthrax bacilli are particularly liable to invade the blood stream either from the pustule or from the lung in wool sorters' disease.

(2) *In certain general infections* the causative organisms may be found in the blood at some stage, usually the early stage, of the disease. Important examples of this form of septicæmia are typhoid and the pasteurella infections, pneumonia, the brucella infections, meningococcal meningitis, particularly during epidemics and in the fulminating cases, and in certain spirochætal diseases such as Weil's disease and rat-bite fever.

(3) *Rigors* occurring during the course of any bacterial infection are the clinical expression of an irruption of organisms from some local nidus into the blood stream. The period of the rigor and for a few hours after is the most favourable time to detect bacteria in the blood and in such infections as those set up by the colon bacillus the blood is almost invariably sterile except at the time of a rigor.

(4) *Infective endocarditis* is a morbid state in which bacteria are present in the cardiac vegetations and are at times demonstrable in the blood. The complete proof of an infective as opposed to a simple endocarditis rests during life upon the recovery of the causative organism from the blood. Unfortunately in a considerable percentage of cases and particularly in the chronic type of the disease with low fever, negative blood cultures are the rule. In cases with high fever, rigors or infarcts, positive cultures can nearly always be obtained and the blood should be taken at the height of the fever or if possible during the rigor period. The organism usually obtained and especially in the post-rheumatic cases, is *Streptococcus viridans* (p. 97). Other varieties of streptococci, some of which may be hæmolytic are also met with. *Pneumococci* are a less frequent cause of infective endocarditis, while *enterococci* and numerous other bacteria have been from time to time reported.

The value of the results obtained from blood cultures. In infective endocarditis a positive blood culture completes the diagnosis, but a negative result has little significance. Vaccines prepared from positive cultures are of no direct benefit to the patient, but in the less acute cases a series of small blood transfusions—from 50 to 200 c.c. of blood at a time—from a donor immunised to the infecting organism has appeared to be of temporary benefit in some cases. In local lesions the cultivation of organisms from the blood stream is of serious import, but a considerable proportion of such cases, in which streptococci or staphylococci are grown, make a good recovery.

when the local condition has been dealt with. The identification of the infecting organism enables the appropriate anti-serum to be used and autogenous vaccines are sometimes of value. In general diseases associated with septicæmia such as the enteric fevers blood culture is the earliest and the most certain means of diagnosis.

In estimating the significance of organisms obtained from the blood it must be realised that the bacteria present in the culture tubes do not necessarily come from the circulation. *Skin contaminations are not extremely infrequent in skilled hands.* They are almost the rule if great care is not taken with the technique. The organisms most frequently obtained in contaminated cultures are staphylococci particularly *S. albus* diphtheroid bacilli and *bacillus subtilis*.

The mode of performing a blood culture. The materials required are a sterile syringe and needle culture media a tourniquet sterile swabs and a towel a bandage and an alcoholic solution of iodine.

The *syringe* should be an all glass instrument capable of holding 10 c.c. The needle should be a moderately stout one with a sharp point and a short bevel. It is as well to be provided with two needles and to test the permeability of both before sterilising. The plunger is removed from the barrel of the syringe and the two parts are separately wrapped in cotton wool to prevent bumping placed in a steriliser or ordinary saucepan filled with water and boiled for half an hour. The syringe is picked out with sterile forceps while the water is still hot and wrapped with the needles in a sterile towel. The syringe will be dry when required for use. The needles are separately sterilised by placing in a beaker on a layer of cotton wool covering with methylated spirit and boiling for 3 minutes.

The *culture medium* requisite for most purposes consists of ordinary beef broth and 4 tubes are required or as an alternative 1 tube of broth 1 of litmus milk and 1 of minced beef medium.

The *iodine solution* is the same as that used before ordinary surgical operations and consists of a 2 per cent solution of iodine in rectified spirits of wine.

The blood is obtained as follows —The patient should be lying in bed with the selected arm supinated fully extended and drawn well away from the side but resting on the bed or

on a pillow, and with the face turned towards the opposite shoulder. Fasten the tourniquet tightly round the arm in such a way as to compress the main vessels and tell the patient to clench his fist. The tourniquet is conveniently a piece of stout rubber tubing 2 feet long. Holding one end in the left hand with the right hand stretch the tubing round the upper arm and exerting traction all the time pass the end held in the right hand round the end held in the left hand and then loop it back under the taut tubing round the patient's arm. To release the tourniquet pull on the looped end. Choose the largest vein about the bend of the elbow—this is almost invariably the median basilic. The vein can be readily seen and felt in almost all subjects but occasionally in young well nourished women it is possible only to feel the vein. If the arm is cedematous the vein may be neither seen nor felt, and in such circumstances it is advisable to expose it as for an ordinary venesection. When the vein has been rendered prominent place a piece of waterproof sheeting under the arm and paint a considerable area of the skin over and round the vessel with the iodine solution. Wash the hands thoroughly and surround the patient's arm with a sterile towel. Take the syringe with the needle firmly attached and pass the needle slowly and steadily through the skin into the vein holding the syringe with the needle pointing in the direction of the blood flow and the barrel of the syringe as nearly parallel as possible with the patient's forearm. Withdraw 10 c c of blood release the tourniquet and remove the needle and syringe. Immediately press a sterile swab upon the puncture mark. Divide the blood among the 4 broth tubes placing 1 c c in the first tube 2 c c in the second 3 c c in the third, and 4 c c in the fourth. It is found that by varying the proportions of blood and medium in the culture tubes a growth of the organisms is more certainly obtained and it not infrequently happens that growth only takes place in the tube containing the least blood. Before leaving the patient paint the puncture wound with iodine and if it is still oozing cover with a piece of sterile gauze and bandage it.

The culture medium employed is commonly broth but this must naturally be varied with the nature of the organism sought for. In infective endocarditis it is often preferable to use the three different media previously mentioned since some streptococci grow poorly in broth and others prefer partial anaerobic

conditions which are provided in the minced beef medium. The media should be incubated at 37°C for 24 hours when a sub-culture is made from each broth tube on to an agar slope and the tubes are again incubated. It is an additional advantage to put up one or two of the original culture tubes under anaerobic conditions. The majority of organisms grow in from 1 to 2 days but some of the streptococci found in cases of infective endocarditis grow very slowly, and all cultures should be kept at least 7 days before they are finally pronounced to be sterile. Even if no visible growth be seen in the broth it is advisable to make and examine films and to sub-culture at intervals on to solid media. The organisms present, having been obtained in pure culture should be examined as to their nature by the ordinary methods.

Tuberculosis The methods of demonstrating the tubercle bacillus differ with the nature of the suspected material. In the case of *sputum* it is advisable to collect a mixed sample of the matter expectorated in the 24 hours. When the *sputum* is scanty or the tubercle bacilli are few in number, the early morning *sputum* should be particularly examined. Quite young children commonly swallow the *sputum* and it may be impossible to obtain sufficient for examination. In such cases the bacilli may be demonstrated in the faeces. A more certain method of detecting pulmonary tuberculosis in a young child is to perform gastric lavage in the early morning on the fasting stomach. The washings are centrifuged film preparations are made and a guinea pig is inoculated.

Sputum is emptied into a wide mouthed bottle provided with a well fitting cork. Five times the volume of 1 in 20 carbolic acid is added and the mixture shaken thoroughly for about 5 minutes. Then stand till the next morning. After standing pour off the layer of mucus which has separated off at the top of the bottle together with the supernatant fluid leaving the deposit of pus. Centrifuge the deposit. Shake the sediment on to a glass slide held in the left hand. Take a second glass slide in the right hand, holding each slide at its extremity. Press the top slide with a to and fro movement several times firmly over the under slide until an even film of *sputum* is spread over both slides. Wipe the backs of the slides dry the films and stain them.

The slides should be examined under the oil immersion lens and at least a quarter of an hour should be spent

on them before the tubercle bacillus is pronounced to be absent

When tuberculosis of the lung is strongly suspected on clinical grounds and the bacilli cannot be demonstrated by this way, the "antiformin" method may be used. "Antiformin" can be prepared by making a stock solution of equal parts of liquor sodæ chlorinatæ (B P) and 15 per cent sodium hydrate. A 20 per cent dilution of this in tap water is shaken with about one fourth its bulk of sputum and left until all the gas has been evolved and a fairly homogeneous solution has resulted. The mixture is then centrifuged at a high speed. Films are made from the sediment and stained in the usual way.

The effect of the antiformin is to dissolve almost everything in the sputum except the tubercle bacillus, the waxy constituents of which are not acted upon.

Cultures can be made from the sediment after washing it free from antiformin with distilled water or animal inoculations may be carried out.

In the case of *urine*, collect the deposit of the urine passed during the 24 hours. Treat the deposit with carbolic acid and prepare films in the same manner as with the sputum. The advantage of investigating the deposit obtained in this way rather than that from a catheter specimen is that the bacilli are less likely to be missed since they are not necessarily passed with every sample of urine. The disadvantage is that smegma bacilli are likely to be present. The smegma bacillus is usually decolorised if the films are passed through alcohol. In the case of all urinary pus, therefore after decolorisation in acid, wash in water and then soak in methylated spirit for 2 minutes. Then wash in water and counter stain in methylene blue.

The examination of urinary slides should be made with particular care, since numerous erroneous diagnoses of tubercle bacilli have been made. The error lies as a rule in mistaking, not the smegma bacillus, but the bacilli of the colon group, which may be present and stained red. The colon bacillus is in no sense acid fast, but it is not infrequent to find that urinary crystals, or even large epithelial cells, have held the fuchsin stain in the acid, and that the stain diffuses out over a small area in the tap water stage and stains the bacilli in that area a bright red. Such bacilli are seen to be lying on

a red background and the mistake is thus easily recognised. The slide must be again decolorised. The red tubercle bacilli must be seen to lie on a blue background. Acid and alcohol fast bacilli may also be found in small numbers in new glass ware, in tap water and in other reagents. These bacilli are usually shorter and stouter than the tubercle bacillus and are as a rule not beaded. In all cases of uncertainty guinea pig inoculation should be practised. In the case of *faeces* tubercle bacilli are commonly very scanty except when actual ulceration of the gut is present. It is advisable to take a small quantity of the *faeces* and treat it with antiformin. Films are made from the centrifuged deposit and stained in the same manner as for sputum.

In the case of tuberculous *pus* such as may be obtained from a caseous gland, a tuberculous joint etc. the bacilli are sometimes numerous but in the great majority of cases are extremely scanty. Such material is best treated at once with antiformin and scanty bacilli can frequently be demonstrated in this way. Antiformin gives better results with tuberculous *pus* or tissues than with sputum.

The detection of tubercle bacilli in pleural, peritoneal and cerebro spinal fluids is dealt with in the section which treats of these exudations.

The *cultivation* of tubercle bacilli is rarely successful if attempted direct from the human lesion owing to the slow growth of the bacillus and the frequent presence of other readily growing organisms. nor is it often necessary to cultivate the bacillus since in the great majority of cases it can be recognised in film preparations. When a culture of the organism is desired the sputum, urinary deposit or caseous *pus* is first inoculated into a guinea pig and the cultures are made from the resulting lesion in the animal. A less certain method is to treat the body fluid with antiformin, wash away the antiformin with sterile saline and then put up cultures from the centrifuged deposit. Cultures may be made in glycerine broth or on Dorset's egg medium but the liquid egg medium of Besredka (p. 250) is advised. It is easily prepared and growth is rapid, a granular deposit of tubercle bacilli appearing within 3 to 5 days.

The *inoculation* of animals may be necessary in order to prove the tuberculous nature of a body fluid in which the bacilli can neither be found nor cultivated or to identify the

exact nature of the acid fast organisms present. The material for inoculation is preferably injected into the thigh of a guinea pig but if the bulk is too great for intramuscular injection the inoculation is made into the peritoneal cavity. About 10 days after injection into the thigh the inguinal gland becomes palpably enlarged. In from 2 to 3 weeks the spleen becomes infected and the animal dies of generalised tuberculosis in from 8 to 10 weeks after inoculation. The injections are made with strict aseptic precautions and preferably two animals are inoculated. After killing the animal the bacilli are readily identified in the more advanced lesions which show the typical gross and microscopical changes of tuberculosis.

Syphilis. The method of obtaining the material for examination has been described in the section on the *S. pallida*. The fluid is examined in one of the following ways —

(a) The Indian ink method. The most suitable variety of Indian ink is that known as chin chin liquid pearl. The bottle must not be shaken.

Place at one end of a clean slide a small drop of the ink.

Take a platinum loop of the exudate and thoroughly mix it with the ink. With a second slide make a thin film of the mixture in the same manner as in making a blood film.

When dry examine direct with the oil immersion lens using artificial light. The spirochaetes are seen as white refractile threads on a brownish black background.

It is difficult to get a good preparation by this method and spiral breaks in the ink film are often mistaken for spirochaetes. It is the simplest but much the least satisfactory method.

(b) By Giemsa's stain. Make on clean slides two or three films of the secretion with a platinum loop.

When dry cover with absolute alcohol and leave for 15 minutes.

Make up from the stock Giemsa's stain a mixture of 9 c.c. of distilled water and 1 c.c. of the stain.

Pour off the alcohol and cover with the stain for 45 minutes.

Wash in a rapid stream of distilled water.

Dry and examine with a $\frac{1}{2}$ inch objective.

The *S. pallida* stains a rose pink colour, other spirochaetes and bacteria blue. The difference in shade between the syphilitic and other spirochaetes is frequently far from obvious and greater reliance is to be placed on the morphological points of distinction.

(c) By Fontana's method *Spirochaetes* are much more readily seen in films treated as follows —

Dry film in air without heat

Fix for 1 minute, renewing fluid twice in the following mixture 1 c.c. acetic acid 20 c.c. commercial formalin 100 c.c. distilled water Mordant in a 5 per cent solution of tannic acid in a 1 per cent watery solution of carbolic acid Heat in this solution until the steam rises for $\frac{1}{2}$ minute Wash 15 to 30 seconds in tap water Do not dry Stain in 0.25 per cent solution of silver nitrate in distilled water to which immediately before use just sufficient ammonia has been added to produce a slight turbidity Heat till steam rises and leave for $\frac{1}{2}$ minute

Wash in water Blot dry Mount

Spirochaetes are stained black

(d) The dark ground illumination (the ultra microscope) A special condenser (the paraboloid condenser) is used for this method of investigation It is fitted into the collar prepared for the ordinary condenser A 'stop' is also used which fits into the inside of the $\frac{1}{4}$ inch objective Special thin slides and cover glasses should also be obtained The illuminant must be a powerful one and should either be a small arc or a Nernst lamp the rays from which should be made parallel by means of a water bottle condenser and projected on to the plane mirror of the microscope The microscope should be vertical

A drop of the fluid is placed on the centre of the slide and if only a small quantity is available saline should be added

The cover glass is carefully let down on to the slide so that all air bubbles are avoided

A drop of cedar oil is placed on the upper surface of the condenser and another on the under surface of the slide The condenser is racked up until the two drops coalesce

A third drop of oil is placed on the upper surface of the cover glass and the objective is focussed after first directing all the available light through the preparation

Instead of an immersion lens the $\frac{1}{4}$ inch objective and a high eyepiece may be substituted

The *spirochaetes* show as shining white refractile bodies on a dark background
 The advantage of this method is that the motility of the bacilli can be studied and it is the method of choice if a

sufficient number of investigations are made to warrant the permanent assemblage of the apparatus

Gonorrhœa In the male the recognition of the gonococcus in a purulent urethral discharge is commonly a simple matter and in the very great majority of cases the causative organism of an acute urethritis is the gonococcus. Occasionally some other organism is the cause of the inflammation and in some cases a discharge of pus from the urethra has an external origin as for example a prostatic abscess and may be caused by the colon bacillus or a staphylococcus. In cases of chronic gleet so long as there is a considerable urethral discharge gonococci commonly remain fairly numerous. Gonococci may in exceptional cases produce a urethritis associated with the causative organism which lasts for years. Not infrequently a history will be given of a urethritis which cleared up some years previously and which has again recurred. In such a case if a thick purulent discharge loaded with gonococci be found it is extremely probable that the patient has exposed himself to a second infection. The latency of the gonococcus in the urethra is a most important question and the examination of the urethra for evidence of such infection is a procedure requiring the co operation of the surgeon and the pathologist. If a slight morning discharge is present or if pus cells and prostatic threads are found in the morning specimen of urine or even if the patient can detect no discharge at all the urethra may still be infective. In all cases in which the patient seeks advice as to infectivity and particularly with a view to matrimony a thorough search has to be made for the gonococcus as well as for clinical evidence of urethral inflammation. The patient should come for examination in the early morning under instructions to hold his water until he has been investigated. The orifice of the urethra should be first examined and if any discharge is found film preparations should be made from it and cultures taken on serum agar. The urine should then be passed and examined by the naked eye for prostatic threads. The urine should then be centrifuged and the deposit searched for pus and epithelial cells and if these are present gonococci should be carefully looked for. The anterior urethra should next be thoroughly irrigated with sterile water. The patient should then be placed on his hands and knees and vigorous massage of the prostate performed. Several minims of clear or turbid prostatic fluid can usually be made to flow

out from the urethra, and this is collected and examined for the presence and nature of the cells and organisms. The urinary examination alone for gonococci should never be depended upon, since it is only exceptionally that gonococci can be recognised in a urinary deposit. If a purulent morning discharge is absent, if there is no pus in the urine, and if the prostatic fluid is clear and contains only mononuclear cells the patient, in the absence of clinical signs or symptoms is probably free from gonorrhœa. If any of these abnormalities are present the gonococcus may or may not be found. All cases of residual urethritis or prostatitis after gonorrhœa are by no means due to the gonococcus. Failure to find the gonococcus at one examination does not, however, exclude the possibility of infectivity. A further search should be made and conducted on the same lines but after the passage of a large sized sound and the irrigation of the urethra with strong silver solution. The inflammatory cells resulting from these procedures should be searched for gonococci. If no gonococci are found the patient is as certainly free from infection as a reasonably careful investigation can prove.

The examination of the female genito urinary tract for gonococci is less commonly successful, even in the acute stage, and greater reliance should be placed on the cultural than on the film results. Before pronouncing a woman free from infection at least three tests should be made. Films and cultures should be taken from the interior of the urethra from the cervical canal after passing a speculum, from the vagina and if clinically infected, from Bartholin's duct.

The culture tubes used should, if possible, be kept warm in the incubator until required and replaced as soon as possible after inoculation. After 24 to 48 hours' incubation, any small translucent colonies should be picked off and stained by Gram's stain. After taking sub-cultures, if required, the tube can be treated as follows. Make up a 1 per cent solution of dimethyl para phenylene diamine hydrochloride (British Drug Houses) in distilled water. Gently wash the surface of a slope culture with about $\frac{1}{2}$ c c of the reagent. Within a few minutes the gonococcal colonies become pink and in half an hour black. The reagent is extremely useful for detecting gonococcal colonies in mixed cultures from the urethra. Film preparations can be made from the stained colonies, but the bacteria are killed.

Diphtheria The taking of swab and culture for the identification of the diphtheria bacillus and the differentiation of this organism from similar bacteria have been described under the heading of the diphtheria bacillus

An alternative culture medium to blood serum is the potassium tellurite medium of Douglas (p 250) After 8 to 12 hours' incubation, the diphtheria colonies are black and those of other bacteria almost colourless Later all colonies become black, but the medium is of definite assistance in obtaining the organism in pure culture

The examination of the culture is conducted as follows — Twelve hours' incubation at 37° C is sufficient to obtain a growth of the organism After incubation examine the colonies with the naked eye and with a hand glass

Make a thin film from the most suspicious colonies Dry and fix the slide

Pour on Löffler's methylene blue and leave it for 3 minutes

Wash in tap water Blot dry and mount

Löffler's methylene blue consists of an alcoholic solution of the dye with potassium hydrate added The beading of the bacillus is more satisfactorily displayed by means of this stain than with the ordinary dyes such as carbol thionin

The identification of the bacillus on morphological grounds is a matter requiring practice and the student is advised to supplement the simple stain by other methods The bacilli are Gram positive, and, further Neisser's method of staining may be used to distinguish the diphtheria bacilli from other organisms, but it must be remembered that certain of the diphtheroid bacteria cannot be differentiated by this means The following is a modification of Neisser's stain —

Make a mixture of 2 parts of a solution composed of

Methylene blue powder, 1 gram ,
Absolute alcohol 50 c c ,
Glacial acetic acid, 50 c c ,
Distilled water, 1 000 c c

and 1 part of the solution

Crystal violet, 1 gram ,
Absolute alcohol, 10 c c ,
Distilled water, 300 c c

Stain in this mixture for 2 minutes Wash rapidly in water.

Counter stain for 15 seconds in the following solution —

Cresoidin 1 gram

(dissolved in 300 c c of warm water and filtered)

Wash in water Dry and mount

The body of the bacillus is stained brown and the granules blue

The bacterial investigation of viscera It is occasionally necessary to investigate the bacterial content of a closed viscus such as a cyst or a pyosalpinx The procedure is as follows —

Place the specimen on a clean plate Heat a metal spatula to red heat in the flame and smear it firmly over the surface of the specimen With a sterile knife cut through the seared surface into the centre of the specimen Hold the edges of the cut apart with a pair of sterile forceps With a platinum loop make films and cultures from the pus or from the fluid in the viscus

The bacteriological examination of viscera *post mortem* is conducted in the same manner In cases of general infection and particularly in typhoid fever the causative organism may be obtained in pure culture from the spleen juice Owing to the rapid emigration of organisms from the intestinal tract into the viscera at the time of death *post mortem* bacterial findings should always be accepted with reserve

Enumeration of bacteria An estimate of the number of organisms present in such substances as pus or faeces can be made by mixing a measured volume (e.g. 0.1 c c) of the material with 10 c c of melted gelatin or agar pouring out in a Petri dish incubating and counting the number of colonies which develop A convenient method for estimating the number of bacteria in fluids such as water milk or urine is the following Take 10 sterile broth tubes each containing 9 c c of media With a sterile pipette add 1 c c of the fluid to tube 1 Shake and mix well With a fresh pipette take 1 c c of tube 1 and add to tube 2 and continue with the remainder of the tubes The sample must be taken and the dilutions made at the source and before any multiplication of bacteria can have occurred The tubes are incubated for 3 days The last tube in which growth occurs gives the number of bacteria per cubic centimetre If growth occurs up to tube 6 the number is 100 000 All tubes in which growth occurs should be plated on agar and MacConkey's medium to determine the type of organism present

Anaerobic cultures By an anaerobic culture is meant the incubation of an organism in an atmosphere free from oxygen. The simplest method of obtaining such an atmosphere in liquid media is to boil the medium in order to expel the air and while still hot to pour on to the surface of it a layer about $\frac{1}{2}$ inch deep of sterile vaseline. The tube is best inoculated with a Pasteur pipette which is passed through the oil along the side of the tube. Care is taken not to introduce air when ejecting the contents of the pipette.

For slope or plate cultures a convenient apparatus is the anaerobic jar of McIntosh and Fildes. It is used as follows. The cultures are placed in the jar. The palladium chloride asbestos is heated to a red heat and the lid with the tap closed is rapidly screwed on to the jar. The tap is opened and connected by pressure tubing with a hydrogen cylinder. When the water ceases to form in the jar the asbestos ceases to glow and the jar cools. The tap is then shut off and disconnected. The apparatus must be used with care.

Animal inoculation Animal inoculation is resorted to for purposes of testing the pathogenicity of an organism or for isolating it or for determining its nature. The animals most commonly employed are guinea pigs, mice and rabbits. Animal inoculation is commonly practised also as a diagnostic procedure in those infections in which the causative organism cannot be detected by other methods. Thus in the spirochaetosis of infective jaundice injection of the patient's blood into a guinea pig is followed by death of the animal in whose viscera the spirochaetes can readily be detected. In the case of many virus diseases animal inoculation may be the only means of diagnosing and of propagating the infective agent. Inoculations should always be carried out with ordinary surgical precautions as to cleanliness of the skin and sterility of the instruments used. Intraperitoneal injections should be made in the mid line high enough to avoid the bladder but below the lower level of the stomach. Intravenous injections are most commonly given into the ear vein of a rabbit. The marginal vein is chosen and after clipping the hair and cleaning the ear with ether a brisk massage with a swab moistened with xylol will make the vein stand out. The needle attached to the syringe is gently threaded into the vein and a little pressure applied to the piston. If the needle is in the vein there is immediate blanching of the vessel.

and the fluid can be injected. If the needle is outside the vein a swelling appears and either another vein or a different part of the same vein must be selected. Intradermal injections are made in the same way as in man but it is first necessary to prepare an area of skin free from hair. This can be done with a razor after clipping the fur or with a depilatory paste. The first method is apt to leave scratch marks and the second method to cause an inflammatory reaction in the skin. An alternative way is to pluck the hairs, a process attended by remarkably little disturbance to the animal.

Filtration. In bacteriology filtration is made use of for —

- (1) Clarification
- (2) The removal of ordinary bacteria—bacterial filtration
- (3) The separation of one filterable virus from another or the removal of filterable viruses—ultra filtration. This last procedure requires very special technique and will not be described here.

Clarification which has as its object the removal of gross particles and obtaining a clear filtrate is employed in the making of media and as a preparatory step in bacterial filtration. In the former case one makes use of filter paper, gauze, lint or glass wool arranged in a suitable filter funnel whilst the latter is most readily carried out with one of the Schott and Gen Jena glass filters which are made in four porosities: No. 1 100–120 μ , No. 2 40–50 μ , No. 3 20–30 μ , No. 4 5–10 μ .

Bacterial filtration is employed for the sterilisation of sera and serous fluids, the separation of bacteria from their toxins and the separation of the ordinary bacteria from filterable viruses. There are numerous makes of filter candle or filter discs available for this purpose, two types only will be described here, the Seitz filter and the Chamberland filter candle.

The Seitz filter consists of a compressed disc of asbestos wool which is mounted in an apparatus designed for the purpose and made by the Seitz Company (Fig. 22). These discs are made in three sizes—3 cm, 6 cm and 14 cm—and two porosities—K for clarifying and EK for removing bacteria. These filter discs are efficient and cheap. They are used once only and thus the tedium of cleaning the filter after use, necessary in the case of filter candles, is obviated. The Seitz apparatus in which the two parts are held together

by pins and fly nuts is much more satisfactory than the model in which the parts screw together

The Chamberland filter is an example of a porcelain filter candle. It consists of a tube of porcelain closed at one end the upper half of which is glazed. Chamberland candles are made in a variety of sizes and a gradation of porosities from L1 the coarsest—merely a clarifying filter—to L11 the finest. The L1 bis L2 and L3 candles are those most frequently used in bacteriological work.

Technique of bacterial filtration
New filter candles should be washed through thoroughly and tested for gross defects before being taken into use. This is done by forcing air through the candle when submerged in water. The escape of large bubbles at any point of the surface indicates a gross

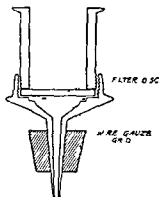


FIG 22—Seitz Filtering Apparatus *

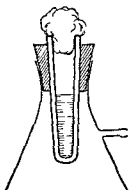


FIG 23—Mounting of Chamberland Filter Candle in Flask *

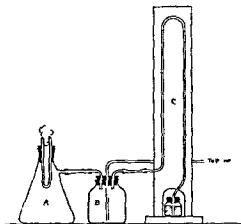


FIG 24—Apparatus assembled for Filtration
A Filter Flask fitted with Chamberland Candle B Trap inserted between Filter Flask Pump and Manometer C Mercury Manometer

leak and the candle should be discarded. The candle or Seitz filter assembled with filter disc in place is mounted in a

* Reproduced from *A System of Bacteriology* by kind permission of the Medical Research Council

filter flask by means of a rubber bung with suitable perforation (Figs 22 and 23). The side arm of the filter flask and the open end of the filter candle or Seitz apparatus are plugged with wool. The filtering apparatus is then sterilised in the autoclave. After sterilisation all joints or fly nuts (Seitz filter) are tightened up—the filter is then ready for use. The filter flask is attached by its side arm to an air pump—Gervik or water pump—by means of pressure tubing. The pump should be furnished with a manometer to control the pressure and in the case of a water pump with a trap consisting of a bottle with rubber bung through which pass two glass tubes. This trap should be inserted between the filter flask and the pump its object being to prevent water being drawn over into the filter flask should the water be turned off while there is still a negative pressure in the flask. The fluid to be filtered is then introduced into the candle or the container above the filter disc in the Seitz apparatus and the fluid drawn through the filter by creating a negative pressure inside the filter flask. Only moderate pressures up to 30 cm. of mercury should be necessary to do this. The filtrate is collected by means of a sterile pipette after removing the rubber bung and filter candle or Seitz apparatus. In all cases it is necessary to know that the filter is bacteria tight. This is ascertained by either adding a suitable test organism such as *B. prodigiosus* to the fluid being filtered and testing for its presence in the filtrate or by filtering a suspension of the test organism after the primary filtration has been carried out. After use the Seitz filter is sterilised in the autoclave taken to pieces and the filter disc thrown away. The parts are then cleaned and reassembled with a new filter disc. The Chamberland candle should not be sterilised by heat after use but should be dropped into 2 per cent. lysol and left there for at least an hour. The filter flask can be sterilised in the autoclave. After coming out of the lysol the candle should be brushed with a stiff brush under running water and treated as follows —

(1) Draw through 20 c.c. 2 per cent. sodium hypochlorite (small candle)

(2) Draw through 100 c.c. $\frac{N}{1}$ HCl and then tap water until the filtrate has a pH of about 7.4

(3) Drain and dry

Certain points should be observed in bacterial filtration —

(1) The least pressure should be used necessary to draw the

fluid through the filter This is usually about 10 cm of mercury

(2) Filtration should be rapid If slow, bacteria may be drawn through

(3) Too great a quantity should not be passed through the filter For the small Sertz disc and the small Chamberland candle the volume filtered should not be greater than 20 c c

(4) Where the object of the filtration is to obtain a filterable virus in the filtrate the pH of the fluid filtered should be about pH 7.6 as in this way loss by adsorption will be minimised Broth is preferable to saline or Ringer as a diluent, as it is known to facilitate the passage of many viruses through the filter

(5) A record of filtration should be kept noting the amount filtered the pH of filtration the time taken the pressure used and the result of the bacterial test

Special staining processes Capsules can usually be seen unstained and in Gram stained preparations appear as a pale space round the bacteria The capsules of pneumococci treated by the specific antiserum stain readily and this reaction forms the basis of a rapid and simple method for typing in sputum, pus or cultures The technique is as follows Mix on a slide 4 loopfuls of Type I serum with 1 loopful of sputum or pus Mount with a cover glass and seal with vaseline Put up similar preparations with Types II and III sera Leave 20 minutes Slide off cover slips and place them in carbolic scrape off vaseline with a knife and allow the film on the slide to dry Wash in tap water to remove the serum Stain with dilute carbol fuchsin 3 minutes, wash and counter stain for 10 seconds with carbol thionin wash and blot dry The cocci are stained almost black the capsules mixed with the homologous serum are swollen and stained red whereas the capsules mixed with the heterologous sera are unaffected and remain as unstained and relatively small clear spaces round the cocci

Spores Fix the film after drying by passing many times through the flame

Stain with boiling carbol fuchsin giving several changes for 10 minutes

Dip in acid alcohol (99 c c alcohol, 1 c c HCl)

Blot dry

Stain in dilute methylene blue 2 minutes

Wash in water Dry Mount

The spores are red and the bacilli blue. The difficulty with the process lies in the differentiation with acid alcohol. With the majority of spores it is difficult to get the fuchsin to penetrate them sufficiently to allow more than the most rapid dipping in the spirit. Successful preparations have quite a striking appearance but it is usually easy to recognise spores in ordinary carbol thionin preparations and to distinguish them from beading of the bacillus by their regular shape and clean-cut outline.

Flagella Film preparations are made from a growth on a solid medium.

In making the films care is taken to avoid picking up any of the medium and to spread the bacteria as thinly and evenly as possible. Prepare the following mordant —

Tannin 2 grams

Water 20 c c

Ferrous sulphate solution half saturated 4 c c

Saturated alcoholic solution of fuchsin 1 c c

Pour mordant over film and heat without boiling 1 minute

Wash in water. Stain with carbol fuchsin

Wash in water. Dry. Mount.

This method is described as the most simple of the flagella staining processes. There are so many cultural and other methods of differentiating the flagellated bacteria that the demonstration of flagella is rarely necessary.

CHAPTER XI

CULTURE MEDIA—STAINING REAGENTS

BEFORE giving the manner of preparing culture media a brief account follows of the changes set up by bacterial growth in the more commonly used media.

Broth is the most universal of all media and in addition forms the basis of numerous others. The majority of organisms growing in broth produce in it a general turbidity and after a time the deposit of a more or less felted mass of bacteria at the bottom of the tube. Some organisms such as the cholera vibrio and certain non pathogenic air bacilli form also a thin pellicle on the surface of the medium. The streptococci on the other hand leave the bulk of the medium as clear as it was before inoculation and form a stringy granular deposit both floating free and attached to the sides of the tube.

These are the appearances obvious in an inoculated tube in addition certain organisms such as the colon bacillus have the property of producing indole from the peptone present in the medium. To test for the production of indole in a broth culture add to the broth a few drops of yellow nitric acid shake the tube and allow to stand. A rose pink colour slowly develops. If the colour is faint add about 1 c.c. of amyl alcohol. Shake and stand the tube until the alcohol separates. The pink colour is extracted by the alcohol. Indole formation may be slow and it is advisable to incubate the tube for a week before making the test. The cholera vibrio produces both indole and nitrites so that the indole test is obtained on the addition of nitrite free sulphuric acid only a reaction which is known as the cholera red reaction.

On examining therefore an inoculated broth tube after incubation one looks for a general turbidity of the medium, a pellicle on the surface, a deposit at the bottom or a clear medium with a granular deposit down the side and then tests for indole formation.

Agar is the most commonly used solid medium just as broth is as a liquid medium. Agar itself is a carbohydrate derived from the stems of certain Chinese seaweeds and agar media

are prepared by adding this substance to broth. The medium is put up either in the form of "slope," "stab," or "plate" cultures. The slope culture tubes are made by pouring a small quantity of melted agar into a test tube, and allowing it to set in a slanting position. The stab cultures are tubes filled or partly filled with the medium, and are inoculated by passing an infected platinum wire into the heart of the medium. They may be used for growing organisms under anaerobic or partially anaerobic conditions. The plate cultures are made by pouring the agar into flat, round, shallow dishes, covered with a loosely fitting lid, and known as Petri dishes.

The majority of organisms grow well on agar, and one is able to tell from the type of colony produced the class of organism present. Bacilli of the coli typhoid group grow in a continuous whitish streak with laterally spreading edges up the surface of an agar slope, and in large, rounded, opaque colonies with thin crenated margins and heaped up centres on plate cultures. Staphylococci produce large, round, opaque colonies with sharp edges, the colonies being white, lemon-coloured or yellow according to the variety of staphylococcus present. Streptococci and pneumococci grow in tiny round translucent colonies barely visible to the naked eye. On examining an inoculated agar culture, therefore, one looks at the type of colonies present, whether they are large or small, white or coloured, translucent or opaque, whether the margins are rounded or crenated, and whether the colonies are discrete or grown together to form a continuous streak. In the case of stab cultures one looks to see if the growth is more abundant at the surface where there is more oxygen, or in the depths of the medium where oxygen is scanty and anaerobic bacteria grow more readily.

Gelatin media consist of broth with sufficient gelatin added to produce a solid medium at room temperature. Since gelatin media are liquefied at 37°C it is necessary to incubate the tubes at or a little above room temperature, that is from 18° to 22°C . Gelatin is put up in slope, stab and plate cultures in the same manner as agar. The most important feature of gelatin as a medium is the fact that some organisms in their growth are able to liquefy it, while others do not. The coli typhoid group of bacilli do not liquefy gelatin and can thus be differentiated from such bacilli as proteus and pyocyaneus, which do. Some organisms, such as the pneumococci, which

are capable of growing on the majority of the usual media do not grow on gelatin. In stab cultures a few bacteria for example anthrax bacilli send out characteristic lateral processes radiating from the line of the stab. A gelatin medium therefore should be incubated at a relatively low temperature and should be examined for the presence and nature of the growth and in particular for the presence or absence of liquefaction.

Litmus milk consists of fresh sterilised milk coloured blue with litmus solution. Litmus milk is a most valuable medium partly because the majority of organisms grow in it abundantly and partly because of the various changes which they may set up in it and by which they may be differentiated. Organisms growing in milk may produce no change in it or may render it more alkaline or more commonly may either simply produce an acid reaction or both acidify and clot it. Other organisms may not only acidify and clot the milk but may eventually decolorise the litmus and further may peptonise the clot and render it liquid again. Litmus milk therefore may be unchanged or may be rendered more alkaline or may be acidified and remain liquid or may be both acidified and clotted.

Litmus carbohydrate peptone water A series of media may be made with peptone water to which litmus is added and 1 per cent of a variety of carbohydrates. The purpose of these media is to test the capability of organisms to break up the carbohydrates present and to produce from them an acid or an acid and gas. The production of acid is shown by the change of colour of the litmus medium from blue to red. In order to observe the formation of gas it is convenient to fill a small tube known as Durham's tube with the medium and sink it upside down in the culture tube so that the gas may collect in the small inverted tube. Numerous carbohydrates are employed in making the media those in fairly common use are glucose lactose mannite salicin and raffinose. In examining the media after inoculation look for the same changes that are to be found in ordinary broth such as the presence of a general turbidity or of a clear medium with a granular deposit and in addition observe the colour of the litmus and the presence or absence of gas in the small inverted tube. The production of an acid reaction is not necessarily accompanied by the evolution of gas.

Neutral red broth consists of broth with a dye called neutral red added. The medium is a useful one for distinguishing organisms and particularly bacilli of the coli typhoid group. The colon bacillus alters the red colour of the broth to a distinct yellow and also produces in the medium a green fluorescence. The typhoid bacillus produces no change other than the general turbidity of its growth.

MacConkey's bile salt medium. This is a solid medium commonly used in plate cultures for the purpose of isolating colonies of the coli typhoid group from mixtures of organisms. The medium has as its basis agar and in addition sodium taurocholate, lactose and neutral red. The presence of the bile salts inhibits the growth of the great majority of organisms other than members of this group. The neutral red gives a different colour reaction with those organisms which ferment the lactose to those which do not. The medium is therefore extremely useful for the isolation of bacilli from such sources as the urine and faeces, since the cocci and other non-pathogenic bacteria do not grow on it, while the colon bacillus grows in bright red and the typhoid bacillus in yellow colonies.

Special media. While the above media are commonly used for organisms of ready growth, a minority of pathogenic bacteria do not grow at all on them or grow very poorly. Numerous special media have been devised for such organisms and a few are in common use. Blood-containing media are essential for the growth of some bacteria and may be obtained by mixing fresh blood with agar or broth. Many delicate organisms, such as pneumococci and meningococci, grow more freely on such media and they are essential for the growth of other bacteria. The gonococcus requires certain constituents of the plasma and the influenza bacillus needs the haemoglobin. Blood agar media are also required for the investigation of the hemolytic properties of bacteria. Blood serum may be used alone in the form of slope cultures after inspissating the serum by heat and is invaluable for the growth of the diphtheria bacillus, which in cultures taken from the throat multiplies rapidly and outgrows the common cocci. The tubercle bacillus is an example of another organism which will not grow on the ordinary media but will grow in broth or on agar to which glycerin has been added. Another useful medium for the growth of the tubercle bacillus is made from eggs and is perhaps the most commonly used medium for this

purpose, both solid and liquid media of this nature are in use

THE PREPARATION OF CULTURE MEDIA

Sterilisation It is essential that all the media and the receptacles which contain them should be perfectly free from all living organisms before they are used for inoculation

The following apparatus is required —

A thermometer graduated to 200° C

A hot air steriliser

A steam steriliser

An autoclave

An inspissator

The *hot air steriliser* consists of a double walled chamber of copper or iron heated below by a gas flame or preferably by electricity and fitted with a thermometer passing into the inner chamber. It is used for the sterilisation of glass flasks, test tubes, Petri dishes, etc. which can thus be rendered both sterile and dry. A temperature of 160° C for 1 hour is sufficient to destroy any organisms that may be present.

The *steam steriliser* consists of a round metal cylinder on legs enclosed in felt or asbestos and provided with a perforated tray fixed about 8 inches from the bottom. Water to the depth of 3 inches is placed in the bottom. The space above the tray should be tall enough to accommodate a litre flask fitted with a funnel. An ordinary potato steamer can be adapted to the purpose. Media placed in the steamer are surrounded with steam at 100° C. The majority of media after being exposed to contamination are steamed for 20 minutes on 3 consecutive days.

The *autoclave* is used for rapid and effective sterilisation by means of steam at high pressure. It is not absolutely essential and requires careful supervision when in use. The autoclave consists of a gun metal cylinder provided with a movable or hinged lid attached by screws and nuts and with a pressure gauge and safety valve. It contains a perforated basket, fitted with legs and a handle. Water is poured in up to the level of the bottom of the basket and the media or other objects for sterilisation are put in. The lid is then lowered and screwed firmly down. The vent is opened and the gas lit. Steam is allowed to pass out of the vent pipe for about 15

minutes and the vent is not screwed down until all air is out of the autoclave that is until the steam passed through cold water is free from air bubbles. The water is usually boiled at a temperature of 120°C which requires a pressure of 15 lbs to the square inch and 15 minutes at this temperature is usually sufficient. The autoclave must be allowed to cool to 100°C before opening it or blowing off steam. All the above sterilisers should be allowed to cool down considerably before opening otherwise the glass receptacles are liable to crack. They must be thoroughly cleansed periodically.

The *inspissator* is required mainly for the preparation of blood serum media. It consists of a shallow sloped chamber provided with a water jacket and a thermometer. The serum tubes are kept at 60° to 65°C 1 hour each day for 2 days and at 70° to 80°C for 1 hour on the third day to solidify the medium. The top of the apparatus is of glass covered with thick felt which can be lifted for inspection of the tubes in the interior of the chamber.

All the receptacles such as flasks and test tubes used in the preparation of media should be perfectly clean and free from acid or alkali. They should be sterilised preferably before being filled and always after exposure to contamination. They should be first washed through with commercial hydrochloric acid then washed thoroughly in tap water then rinsed in distilled water and dried in the hot air steriliser*. Flasks and tubes are then plugged with non absorbent cotton wool and packed in wire crates for sterilisation. The plugs should fill the mouths of the tubes and should project beyond them but should not be too tight nor too bulky. Ordinary cleanliness should be observed in all operations connected with media making in order to avoid the needless introduction of spore bearing organisms. The methods of making the following media are given in brief and the smaller details are left to the management of the individual worker.

Standardisation The reaction of media is now almost universally adjusted on the basis of the hydrogen ion concentration or pH. The method of standardisation is as follows —

Materials required micro burette 5 c.c pipette cordite tubes (namely tubes of neutral glass and standard bore)

* Tubes and flasks are preferably made of a neutral glass such as Monax. The cheaper glass even after thorough treatment will still give an alkaline reaction to phenol red.

$\frac{N}{10}$ and $\frac{N}{1}$ caustic soda $\frac{N}{10}$ and $\frac{N}{1}$ hydrochloric acid, distilled water, and, as indicator, 0.04 per cent phenol red in distilled water. Also a special stand for 4 tubes and a set of standard tubes ranging from pH 7 to 8. The standard tubes can be purchased. The stand is a square one, boxed in at the sides, with holes at the top for the tubes and observation slots, about $\frac{1}{2}$ inch wide, front and back.

After broth medium has been rendered alkaline to thymolphthalein, boiled for 3 minutes and filtered to remove the phosphates, the reaction is adjusted by adding normal hydrochloric acid to bring it to pH 7.8. To effect this refer to the diagram on p. 323 and the list of standard solutions on p. 257. The tubes are here referred to as C and D, left to right of back row, and A and B of front row. Proceed as follows—In tube C put distilled water; in tube D 5 c.c. of medium; in tube A 5 c.c. of medium and 0.25 c.c. of indicator (phenol red). In tube compartment B put standard tube of required pH. Run into A from a burette $\frac{N}{10}$ acid until a match is made, on holding the stand up to the light, with the standard in B. Read the exact amount of $\frac{N}{10}$ acid used for the 5 c.c. of the medium and add the requisite amount of normal acid to bring the litre to the correct pH.

To standardise a solid medium, such as agar, dilute the melted agar 1 in 3 with boiled distilled water; add the indicator while hot, cool, and then take the reading.

Stock nutrient broth. This medium is widely used and forms the basis of many other media.

Procedure. Mince 1 lb. lean fresh steak, freed of fat. Add 1 litre of distilled water and stand in ice chest overnight. Boil for 15 minutes and filter through gauze or glass wool. Add 1 per cent peptone and 0.5 per cent sodium chloride and, when dissolved, make alkaline to thymolphthalein (see under standardisation). Bring to boil and filter through Chardin paper to remove phosphates. Cool and standardise to pH 7.8. Tube, or place in 500 c.c. flasks for stock. Steam for 20 minutes on 3 successive days, or autoclave for 15 minutes at 15 lbs. Sterilisation by steam is preferable to autoclaving.

Broth of double strength, made by adding 500 c.c. of water

instead of 1 litre to the above ingredients is advised for the preparation of agar media

Glycerine broth is made by the addition of 6 per cent of glycerine to the stock nutrient broth

Glucose broth is made by the addition of 2 per cent of glucose to the stock nutrient broth It must be sterilised by steam

Neutral-red broth is made by adding 2 c c of a 2 per cent aqueous solution of neutral red to 100 c c of nutrient broth

Litmus carbohydrate peptone water is made by adding 1 gram of the carbohydrate to 90 c c of peptone water * of pH 7.8 and 10 c c of litmus solution The carbohydrates most commonly required are dextrose lactose mannite raffinose and salicin Others which may be employed are arabinose saccharose mulin coniferin, sorbit etc

The litmus solution required to give the necessary colour may be bought ready made or prepared as follows —

Materials required—

Litmus powder	20 grams
90 per cent alcohol	200 c c
Distilled water	200 ,

To make —

Boil the litmus with 80 c c of the alcohol for 1 hour on a water bath using a reflux condenser

Pour off the clear liquid

Repeat with 60 c c of the alcohol

Repeat with the remainder of the alcohol

Digest the washed litmus in the distilled water

Filter

When tubing the carbohydrate media it is convenient to place in each test tube a small inverted tube filled with the medium in order to collect the gas which may be evolved as the result of bacterial growth

Agar-agar Agar medium consists of a solution of agar agar in stock nutrient broth It is made as follows —

Add 25 grams of agar powder to 500 c c of distilled water and dissolve in steamer or autoclave Add 500 c c double strength broth of pH 7.8 Cool to 50° C and add beaten whites of 2 eggs Steam for 30 minutes and filter clear through Chardin paper Adjust pH if needed and pour in tubes or flasks Steam or autoclave

* One per cent sugar free peptone half per cent sodium chloride in water

The tubes should be prepared in two forms—for “stab” cultures, in which case the tubes are filled to about two-thirds their capacity and allowed to cool in the vertical position, and for “slope” cultures, in which case they are filled about one sixth full and allowed to cool in a slanting position

MacConkey's neutral-red agar. This medium is made as follows —

Dissolve “Lab Lemco ”	10 grams
Peptone	10 „
Sodium chloride	5 „

in 1,000 c c of water Add 25 grams of agar powder Dissolve in steamer Cool and clear as for agar Standardise to pH 7.8 Add lactose 1 per cent, sodium taurocholate 0.5 per cent and 2 per cent of a 1 per cent aqueous solution of neutral red Tube for ‘stab’ cultures and steam

Serum agar Melt 86 c c sterile agar of pH 7.6 and cool to 50° C Add 14 c c horse serum warmed to 50° C Tube and slope Incubate to test sterility The addition of 1 per cent defibrinated blood is an advantage

Gelatin Gelatin media consist of broth with sufficient gelatin dissolved in it to produce a solid medium at temperatures from 18° to 22° C The necessary proportion of gelatin to broth is 25 per cent of the former during the summer and 20 per cent during the winter It is important to obtain the best gelatin, otherwise a soft medium will result Coignet's gold label gelatin is very satisfactory

To make —

Add the gelatin to warm nutrient broth

Dissolve while warm

Standardise at pH 7.8

Add beaten white of egg, to clear, as with agar

Filter

Tube as required (Put up both slope and stab media)

Steam Do not autoclave

Litmus milk The milk must be quite fresh, and preferably the greater part of the cream should have been removed

Steam for 30 minutes

Filter

Add 10 to 15 per cent of the stock litmus solution

Tube

Steam the tubes for 20 minutes on 3 consecutive days

Potato medium This medium is much less commonly

used than formerly. Special test tubes are required, which resemble Buchner's anaerobic tubes in miniature. A few drops of glycerine are placed in the bottoms of the tubes and sticks of potato well washed are cut and passed into the tubes, which are plugged with wool and steamed for 1 hour on 3 consecutive days. Unless the tubes are used at once, it is preferable to cover the wool plugs with rubber caps.

Dorset's egg medium This medium is used for the growth of the tubercle bacillus, and is prepared as follows —

Four eggs are well beaten up. 25 c c of water are added, and the whole is thoroughly mixed. Add 6 per cent glycerine. The mixture is passed through muslin and tubed. Sterilise as for serum tubes.

Besredka's liquid egg medium Carefully remove the yolks of four eggs and beat them up with 200 c c of freshly distilled water. Run in slowly 35 c c of 1 per cent NaOH, stirring all the time. Make up the clear egg solution to 1,400 c c, with distilled water. Filter through gauze. Autoclave at 15 lbs for 20 minutes.

Blood serum media Blood, preferably that of a horse, is obtained at the slaughter house, and is taken from the wound, after a little blood has been allowed to flow, direct into a large sterile glass cylinder. The cylinder is placed in the cold until the serum has fully separated, that is, as a rule, until the next day. The clear serum is then removed with a sterile pipette placed in 100 c c well stoppered glass bottles, and chloroform is added to 0.25 per cent. The stoppers are tied down and the bottles incubated for 3 days and shaken occasionally. The sterility of the serum is tested and it is stored in the cold.

For Löffler's blood serum 1 part of nutrient broth of pH 7.8 is mixed with 3 parts of the serum and 1 per cent of glucose. The tubes are then inspissated in the sloped position (p. 246).

For Douglas' serum and potassium tellurite agar medium for diphtheria bacilli, trypsinised horse serum is used, and 5 to 8 c c of Allen and Hanbury's liquor trypsin co are added to 100 c c of the serum chloroform mixture. After incubation of the serum as above, 100 c c of neutral agar are melted and mixed with 1 c c of 1 per cent potassium tellurite, cooled to 50° C, and 15 c c of the serum are added. The mixture is poured into tubes and sloped, or into Petri dishes.

Instead of horse blood that of the sheep or ox can be used or human blood obtained at venesection

Hydrocele fluid or ascitic fluid can be made use of in the same manner

Blood agar To neutral agar melted and cooled to 50°C add 4 per cent of defibrinated human or rabbit blood

Ox bile medium This medium may be used for the growth of the typhoid bacillus

The gall bladder of an ox is tied off and removed at the slaughter house 100 c c of bile are mixed with 10 grams of peptone and 2 grams of sodium chloride

The mixture is autoclaved for 15 minutes at 120°C filtered and tubed

Gonococcus medium Mince bullock's heart, free from fat and fibrous tissue and add tap water in the proportion of 2 c c to 1 gram of the minced heart Stand 48 hours in ice chest Filter and squeeze through gauze Add 1 per cent peptone and 0.5 per cent acid sodium phosphate Steam for 45 minutes stirring at the end of 30 minutes Allow to cool and filter through Chardin filter paper Standardise to pH 7.5 Add 2.5 per cent agar powder autoclave 30 minutes at 15 lbs pressure cool to 50°C and clear with egg white Steam 1 hour and filter Adjust pH Tube and steam for $\frac{1}{2}$ hour Next day steam $\frac{1}{2}$ hour cool to 50°C and add sterile hydrocele fluid 1 c c to 5 c c of medium Slope and incubate for 48 hours to test sterility The hydrocele fluid can be kept in sterile flasks containing 0.5 per cent chloroform

Influenza bacillus medium Take 150 c c saline 6 c c pure HCl 50 c c defibrinated sheep's blood 1 gram pepsin (B.P. granulated) shake and dissolve Place in water bath at 55°C for 18 hours shaking at first Add 12 c c of 20 per cent NaOH and test reaction with cresol red (0.05 per cent) adding NaOH until colour is that of permanganate (pH 7.6) Add HCl drop by drop until cresol red gives no change of colour and phenol red gives red colour (pH 7.0-7.2) Place in sterile stoppered bottle for stock and add 0.5 per cent chloroform To make up mix 5 per cent of stock with neutral agar Tube and incubate to test sterility

Sabouraud's Glucose Medium Tap water 1000 c c Glucose (Brute de Chanut) 40 grams Peptone (granules de Chassaing) 10 grams Agar 18 grams Soak agar in water for 1 hour Add glucose and peptone Autoclave slowly up to

120° C Tube Autoclave up to 120° C slowly For the maltose medium the same amount of maltose is substituted for the glucose

Noguchi's Leptospira Medium 0.9 per cent sodium chloride 800 parts Rabbit serum (inactivated) 100 parts Hæmoglobin solution 0.1 to 0.2 parts 2 per cent nutrient agar (pH 7.2) 100 parts After melting the agar cool to about 45° C Warm the saline serum and hæmoglobin mixture to the same temperature and add quickly to the agar Mix thoroughly The hæmoglobin solution is made by mixing 1 part of defibrinated rabbit's blood with 3 parts of distilled water

The sterilisation of inoculated media It is necessary to destroy the organisms which have grown in the discarded culture media and to render the test tubes and plates fit for further use Tubes and Petri dishes are placed without removing the wool plugs or separating the dishes in an enamel pail or saucepan and covered with water *Lotio Saponis* is added and the pail is autoclaved for 30 minutes at 20 lbs pressure The test tubes are then thoroughly washed with tap water with the help of a test tube brush and rinsed in commercial hydrochloric acid They are then washed thoroughly again in tap water drained rinsed in distilled water dried plugged and sterilised The dishes are scraped clean washed thoroughly in several changes of tap water and sterilised

To clean used microscopic slides Soak the slides in cold water to remove the lysol Boil or soak in a solution of 1.5 per cent potassium bichromate in 10 per cent sulphuric acid Thoroughly wash in tap water and dry with a clean cloth

THE PREPARATION OF STAINING REAGENTS

The number of staining reagents required for the ordinary routine methods of clinical pathology is very small Only the formulæ of the essential stains are given here and it is remarkable how rarely one requires any stain which is not to be found in the scanty array of drop bottles disposable on one small shelf

The stains given here include those required for ordinary histological purposes as well as for bacteriological work The special blood stains are described in the section on the blood and a few other special stains are given in their appropriate places in the text

Carbol-thionin This is the most useful general stain in routine bacteriological work. It has the following formula —

Saturated solution of thionin in 50 per cent alcohol	10 c c
Carbolic acid (crystal), 5 per cent dissolved in distilled water	90 c c

The saturated alcoholic solution of thionin contains about 2 per cent of thionin. Both this and the carbol thionin are apt to precipitate if put into new bottles.

Methylene-blue A saturated watery solution has the following composition —

Methylene blue	about 20 grams
Distilled water	400 c c

The stain is added to the distilled water in a bottle with a well fitted stopper and the bottle is kept in the warm (or in the incubator at 37° C.) for several days. If all the stain is dissolved more is added until the mixture is saturated.

A saturated alcoholic solution is made in the same way by adding about 7 grams of methylene blue to 100 c c of absolute alcohol.

The saturated watery and alcoholic solutions are kept as stock, and a 1 per cent dilution of the watery solution is used for ordinary staining purposes. The alcoholic solution is required for the next stain.

Löffler's methylene-blue Formula —

Saturated solution of methylene blue in alcohol	30 c c
Caustic potash 1 per cent	1 "
Distilled water	100 "

Carbol-fuchsin Formula —

Basic fuchsin	1 gram
Absolute alcohol	10 c c
Carbolic acid 1 in 20	100 "

The stain is shaken with the alcohol and the carbolic added to it. This solution is kept as stock and is filtered into drop bottles as required.

Gentian-violet (alcoholic) Formula —

Gentian-violet	about 5 grams
Absolute alcohol	300 c c

The mixture is kept in the warm or in the incubator for 1

week, and if not saturated at the end of this time more gentian violet is added

This solution is kept as stock and filtered before use

Methyl-violet (6 B watery) Dissolve 0.5 grams in a little absolute alcohol and make up to 100 c.c. with distilled water. Keep as stock. Filter before use

Gram's iodine Formula —

Iodine	1 gram
Potassium iodide	2 grams
Distilled water	300 c.c.

A clear solution results which does not require filtering

The triple strength of Gram's iodine made by dissolving the above amounts of iodine and potassium iodide in 100 c.c. of water is advised for Gram's staining method (p. 216)

Giemsa's stain Formula —

Azur II eosin	3 parts
Azur II	0.8 "
Glycerine (pure)	250 "
Methyl alcohol (acetone free)	250

Grind up the stain with the methyl alcohol adding the alcohol in three or four separate fractions. Then add the glycerine

Safranin This is a useful counter stain to Gram's method for organisms in sections

Formula —

Safranin	0.5 gram
Distilled water	100 c.c.

The stain is filtered on the section where used

Castaneda's stain (River's modification) Formula —

Phosphate buffer M/15 pH 7.0	95 c.c.
Formalin	5 "
Löffler's methylene blue	10 "

Hæmalum This is a good nuclear stain for tissues and is also convenient for the staining of leucocyte drops by Strong's method

Formula —

(1) Pure hæmatem	2.5 grams
Alcohol 95 per cent	95 c.c.

Dissolve

(2) Ammonium alum	125 grams
Distilled water	2 000 c c

Dissolve

Add (1) to (2)

Add to the mixture

Glacial acetic acid	325 c c
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Shake well and allow to ripen on a shelf in the light if possible for from 1 to 3 months

Filter before use

Ehrlich's acid hæmatoxylin

Solution I

Hæmatoxylin	2 grams
Absolute alcohol	60 c c

Solution II

Make a saturated solution of potassium alum in equal parts of glycerine and distilled water Take 60 c c of this and add 3 c c of glacial acetic acid

Mix I and II and leave in the sunlight for 6 weeks

Weigert's iron hæmatoxylin

Solution I

Hæmatoxylin	1 gram
96 per cent alcohol	100 c c

Allow to ripen in sunlight for a few days

Solution II

Liquor ferri perchlor (B P)	2 c c
Hydrochloric acid	1
Distilled water	95

Mix the two solutions in equal parts before use and use within half an hour

Eosin This is an excellent tissue stain for sections

Alcoholic solution A saturated solution is made in methylated spirit About 2 grams are required for 100 c c of spirit Five per cent of this solution in distilled water or spirit is used for staining purposes

Watery solution Distilled water 500 c c

Eosin 7 grams

Van Gieson's stain This stain is a very widely used

differential stain for histological purposes It has the following composition —

Saturated watery solution of picric acid, filtered	100 c c
Watery solution of acid fuchsin 1 per cent	10 „

Scharlach R This stain is the most satisfactory for the demonstration of fat in tissues or in film preparations Fat globules are stained red by it, and all other tissues are left unstained It is prepared as follows —

Make a saturated solution of Scharlach R in a mixture of 50 c c of 70 per cent alcohol in distilled water and 50 c c of acetone

Weigert's elastic stain This is the best stain for elastic fibres in tissue Dissolve 2 grams of basic fuchsin in 200 c c of a 2 per cent solution of resorcin in distilled water Boil this solution in a porcelain dish When boiling add slowly 12.5 c c of liquor ferri perchlor fort B P (or, better, 25 c c of liquor ferrisessquichlor German Pharmacopœia) and boil for 5 minutes stirring with a glass rod A precipitate is formed Allow to cool Filter Throw filtrate away Allow deposit on filter paper to dry Put filter paper and deposit in the previously used porcelain dish, add 200 c c of 94 per cent alcohol and bring to the boil stirring all the time and fishing out bits of filter paper when they are free from stain This takes 3 or 4 minutes Cool Filter Make up filtrate to 200 c c with 94 per cent alcohol Add 4 c c hydrochloric acid This stain lasts about 3 weeks After that it tends to stain the tissue diffusely and not only the elastic fibres, in spite of differentiation

Hart's elastic mixture lasts longer (about 3 months) Take 15 c c (perhaps better, 30 c c) of Weigert's elastic stain that is not more than 3 to 6 months old and make it up to 100 c c with 1 per cent acid alcohol (1 c c of hydrochloric acid in 100 c c of 70 per cent alcohol)

Aniline gentian violet This is used for Weigert's modification of Gram's method for sections

Aniline oil	10 c c
Distilled water	100 „

Shake up for 10 minutes A milky emulsion is formed Filter through a filter paper that has been well moistened with distilled water

To 90 c c of the clear filtrate, which has been allowed to stand for 5 minutes, add 11 c c of a concentrated alcoholic solution of gentian (or crystal or methyl) violet. This solution deteriorates after about 3 weeks.

Mucicarmin for staining mucus in sections. 1.5 grams of mucicarmin in 100 c c of 50 per cent alcohol.

Saturated solutions. In the following solutions the solvent is added to the stain and shaken occasionally for 1 hour until the stain is dissolved. The proportions of stain to solvent are as follows —

Fuchsin in alcohol	.	.	3 per cent.
Gentian violet in alcohol	.	.	1.5 „
Gentian violet in water	.	.	4.8 „
Methyl blue in water	.	.	6.7 „
Methyl blue in alcohol	.	.	7 „
Saffranin in water	.	.	4 „

Standard Solutions

Required $\frac{M}{5}$ acid potassium phosphate dissolve 27.25

grams of pure dry acid potassium phosphate in distilled water and make up to 1 litre. Keep in a hard glass bottle.

$\frac{N}{10}$ NaOH kept in a well stoppered paraffined bottle.

Procedure. Measure 25 c c of the acid phosphate solution into a 100 c c flask add $\frac{N}{10}$ sodium hydrate according to the following table dilute to 100 c c and mix.

pH	NaOH added	pH	NaOH added
7.0	29.63	7.6	42.80
7.1	32.50	7.7	44.20
7.2	35.00	7.8	45.20
7.3	37.40	7.9	46.00
7.4	39.50	8.0	46.80
7.5	41.20		

The solutions thus made are stored in Pyrex bottles with well fitting stoppers in the ice chest and will keep for several weeks.

SECTION III.—PUNCTURE FLUIDS

CHAPTER XII

GENERAL PROCEDURE—PLEURAL FLUIDS— PERICARDIAL FLUIDS

By puncture fluids are meant such body fluids, whether exudates transudates, or the contents of cysts, as are commonly removed for examination by means of a needle and syringe

The fluids most frequently taken for investigation are those derived from the pleural and peritoneal cavities and from the cerebro spinal canal

It is only possible to consider here those methods of investigation which have a direct clinical bearing

GENERAL PROCEDURE

The methods of examination of fluids from different sources will be described for each fluid but the following procedures should be adopted in all cases

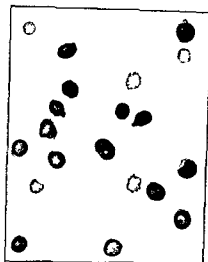
(1) *Inspection* The naked eye appearance is often of great importance and a written record should always be made It should be noted whether they are clear, turbid or flocculent, if they contain blood and, if so, whether the blood is in great or small amount and intimately mixed or not, if a clot forms on standing and the appearance of the clot

In the case of purulent exudates, the examination is conducted in a precisely similar manner to that employed for the investigation of pus from any other part of the body

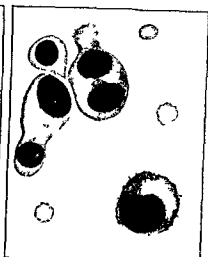
(2) *Chemical examination* It is rarely necessary to make any detailed chemical examination A rough estimate of the protein content is advisable and gives an indication of the intensity of the inflammatory process

(3) *Cytology* By cyto-diagnosis is meant an investigation of the number and nature of the cells present in a fluid Such investigation must never be omitted, and should be made as soon as possible after the fluid has been withdrawn

PLATE IX



Small Lymphocytes.
Tuberculous Pleural Exudate
(Leishman's Stain)



Endothelial Cells
(Peritoneal Fluid - Cirrhosis of Liver)
(Leishman's Stain)



Polymorphonuclear Neutrophils and
Meningococci
(Cerebro-spinal Fluid)
(Leishman's Stain)



Polymorphonuclear Neutrophils and
Pneumococci
(Pleural Exudate)
(Carbol-thionin)

THE CELLS OF PUNCTURE FLUIDS

[Total page 9]

The cells which may be present in these fluids are mostly of three varieties, namely, lymphocytes, polymorphonuclear neutrophils, and endothelial cells. A relative predominance of one of these cells, when present in excess, yields the most definite information of the underlying pathological process.

Lymphocytes in excess are diagnostic of the chronic infective granulomata, of which syphilis and tuberculosis are by far the most important examples. The clinical distinction between syphilis and tuberculosis in the case of the central nervous system is in the great majority of cases clear, syphilis of the pleural and peritoneal sacs is an extremely rare pathological condition, consequently the demonstration of an excess of small lymphocytes in any of these fluids commonly suffices to substantiate a diagnosis.

These lymphocytes, or, as they are called by some, lymphoid cells, cannot be distinguished on histological grounds from the small lymphocyte of the blood, the cell which is relatively increased in the circulating blood in tuberculosis and syphilis. The cell is also probably identical with the lymphoid cells found in the tissues in large numbers in the neighbourhood of tuberculous, and to a less obvious extent of syphilitic, lesions. The part played by the lymphoid cells is obscure, but it is this type of cell which is actively attracted into the blood, and from the blood into the exudates and tissues, by the toxins of the tubercle bacillus and the *S. pallida*.

Polymorphonuclear neutrophils The presence of these cells is definite evidence of an acute inflammatory process. Acute inflammation of the serous sacs or of the cerebro spinal canal may be induced by bacteria or by an aseptic irritant acting as a foreign body. In human pathology the exciting cause is in the vast majority of cases a pyogenic organism. The demonstration of polymorphonuclear neutrophils therefore calls for a further examination by microscopical and cultural methods in order to identify the causative organism. The distinction between clear turbid and obviously purulent fluids all containing the polymorphonuclear neutrophil as the predominant cell is only one of degree. The process has in each case the same pathological basis. For example, a clear or faintly turbid fluid withdrawn from the chest after an attack of lobar pneumonia may be shown on examination to contain both polymorphonuclears and pneumococci, and thus to be of the same nature as the thick pus of an ordinary empyema. When blood

is present the demonstration of an occasional polymorphonuclear neutrophil is of no significance, and one has to judge in such cases whether the leucocytes of the deposit are in excess of what is natural to the number of red cells, remembering that in normal blood the proportion of white cells to red is only about 1 to 1,000

Endothelial cells (Plate IX.) These cells are derived from the lining membranes of the serous cavities, and their presence is evidence of a passive transudate. They are rarely found except in very small numbers, in cerebro spinal fluid. They may be very numerous in pleural or peritoneal fluids. The cells are of much the same character in all situations in the body and consist of large, more or less rounded cells, the cytoplasm of which is basophilic and often vacuolated. The edges of the cells are frequently frayed and irregular. The nucleus contains as a rule two or three well marked nucleoli. The endothelial cells are phagocytic and may contain red cells and in the case of mixed cellular deposits, lymphocytes, polymorphonuclear cells, or bacteria. They often occur in plaques of considerable size. In a good preparation these cells are quite unmistakable, but if the film has been made too thick the cells may be so shrunken as to be almost indistinguishable from small lymphocytes. In such cases the nature of the cells is commonly revealed by examining the edge of the film where the fluid is thinner and the cells have dried more quickly and have consequently shrunk less.

An excess of endothelial cells is to be found in a variety of conditions including renal disease, general cardiac failure and any local obstruction to the circulation, as in malignant disease or cirrhosis of the liver.

Other cells Almost any variety of cell may on rare occasions be found in these fluids. The *eosinophil* cell is exceptionally predominant. It may be found in parasitic effusions, as in the content of a hydatid cyst or after the rupture of a cyst into the pleural or peritoneal cavities. It may form a considerable percentage of the cells present in the clear exudates which occasionally follow lobar pneumonia and is then of good prognostic significance since such cases apparently do not proceed to suppuration.

The large hyaline is never the predominant cell but is not infrequently found in small numbers particularly in association with polymorphonuclear neutrophils.

Malignant cells, whether sarcomatous or carcinomatous, are frequently described and practically never identified. The cells which are sometimes figured as sarcoma cells are either small lymphocytes or quite indistinguishable from them. The cells often described as carcinoma cells are in every way similar to endothelials. It is true that fragments of growth may be washed off into a serous fluid and may very rarely be identified microscopically, but in the great majority of malignant cases the predominant cell in an exudate is not to be distinguished from either an endothelial cell, or much less commonly, a small lymphocyte. A carcinoma or sarcoma cell floating free has no distinguishing marks by which it can be identified.

(4) **Bacteriology** The method of bacteriological investigation varies with the type of cell present in the exudate and consequently a portion of the fluid should be examined cytologically before an attempt is made to discover the presence of a causative organism.

If the predominant cell is an endothelial no bacteriological investigation is necessary, since the presence of bacteria in such fluids must be due to a contamination either from the patient's skin or from the needle and syringe.

When the predominant cell is a polymorphonuclear neutrophil the bacteriological investigation must be conducted on exactly the same lines as that for acute inflammatory exudates in any part of the body. The causative organisms themselves may often be seen in the film preparations made primarily for the identification of the cells and cultures are then made upon the appropriate media. If no organisms are seen a growth may still be obtained in the cultures which should be made on media suitable to the organism suspected from the nature of the case. In a small proportion of acute inflammatory exudates organisms may be seen in the films but owing probably to the previous exposure of the bacteria in the body to bacteriolytic substances the attempt to grow them in culture media fails. In such cases a fairly accurate bacteriological diagnosis can usually be made from the nature of the case and the morphological appearance of the organisms. The pneumococcus in pleural or other exudates and the meningococcus in cerebro spinal fluids not infrequently stain very faintly, and sometimes fail to grow upon the ordinary media.

In another small percentage of cases polymorphonuclear neutrophils are the predominant cell, but no organisms are

seen in the film preparations and none grown in culture. Some of these fluids are in reality produced by pyogenic organisms which have escaped recognition. Others are not the product of organisms but of some mechanical irritation, such as an injury or the presence of new growth, or an effusion of blood acting as a foreign body. Others, again, may turn out to be tuberculous effusions in which the predominant lymphoid cell has degenerated or disappeared and the polymorphonuclear neutrophil, which is acting as a phagocyte of the cellular debris, comes to predominate. Primary tuberculous effusions may further become secondarily infected by pyogenic organisms, and in such cases the underlying pathological process may be entirely missed, unless a knowledge of the clinical condition of the patient suggests that the tubercle bacillus also should be specially sought for.

When the small lymphocyte is the predominant cell there is no object in putting up the ordinary culture media and searching for the presence of pyogenic organisms. Any bacteria which may be seen in the ordinary film preparations or grown on the stock media are evidence of failure in the aseptic technique. When an excess of small lymphocytes with a relative predominance of 80 per cent or more over the other cells is found, a diagnosis of either a syphilitic or a tuberculous infection can be made with a considerable degree of confidence. Such a diagnosis, however, is an indirect one and thus liable to a small but definite percentage of errors, consequently further investigation is preferable whenever practicable. In syphilitic cases a Wassermann reaction should be performed with the patient's serum, or with the fluid, or with both. In the case of tuberculous effusions an attempt should be made to identify the bacillus, but unfortunately in the majority of these fluids the bacilli are extremely scanty and require special methods to display them, such as will be described subsequently.

A small percentage of lymphocytic effusions are neither tuberculous nor syphilitic. Very occasionally miliary growths of carcinoma or sarcoma on the pleural membranes or less commonly on the peritoneum, may act as chronic irritants and produce an exudate rich in lymphoid cells. Such exudates are liable to be mistaken, both on clinical and pathological grounds, for tuberculous exudates. Similar exudates may accompany an aneurysm of the thoracic or abdominal aorta, or an enlarge

ment of the mediastinal glands by new growth or Hodgkin's disease. All these rare pathological states are with the exception of Hodgkin's disease more commonly associated with an excess of endothelial cells and the percentage of error in lymphocytic effusions is very small.

Methods The methods to be adopted in the examination of the various fluids do not materially differ but it is convenient for the sake of clearness to describe the procedure for each fluid.

PLEURAL FLUIDS

All puncture fluids must be obtained with strict aseptic precautions. The patient's skin should be painted with iodine solution and the needle and syringe must have been boiled. The needle should be a fairly long and stout one and the syringe should be capable of holding at least 10 c.c. The place chosen for puncture necessarily varies with the physical signs present but in the majority of cases the puncture is best made midway between the posterior axillary and scapular lines in the ninth or tenth space. If much fluid is present the syringe when full is detached from the needle and emptied into a sterile test tube and the process repeated for a second tube. The fluid withdrawn may be obviously purulent or more or less clear.

Purulent pleural exudates are examined as follows —

(1) Observe whether they are offensive or non-offensive. The majority of these effusions have little odour, a minority stink most evilly. Stinking pus from the chest is of diagnostic significance since it indicates pretty certainly that one is not dealing with a simple empyema, that is with an abscess confined to the pleura. The smell is produced in the great majority of cases by a long thin saprophytic bacillus whose normal habitat is the respiratory or alimentary tract. In a small percentage of cases the smell may be due to or augmented by the presence of coliform bacilli or anaerobic gas forming streptococci. The significance of these organisms is that a communication has taken place between the pleural cavity and the lung or between the pleura and an abdominal viscus. The presence of stinking fluid therefore combined with the information acquired by further examination suggests one of the following alternatives. A primary abscess of the pleura (or empyema) has ruptured into the lung, a primary abscess

of the lung has ruptured into the pleura, an abdominal abscess has ruptured through the diaphragm into the pleura, a bronchiectatic, and not a pleural abscess has been punctured. The prognosis for any of these alternatives is evidently less favourable than for an *uncomplicated empyema*.

(2) Make film preparations. Stain with carbol thionin and by Gram's method. The cells present will be found to consist almost entirely of polymorphonuclears.

Organisms are usually to be seen in addition. The organism most commonly met with is an extracellular capsulated diplococcus with pointed ends, the *pneumococcus*. The great majority of empyemata follow lobar pneumonia, and in most cases the pneumococcus is present in large numbers and in pure culture. The diagnosis of the causative organism can be made with considerable certainty from film preparations in many cases, but it is advisable always to confirm by cultural tests. Numerous other organisms may appear in pairs in pus films, and the pneumococcus often forms short chains of 4 to 8 members.

The organisms found next in order of frequency are *streptococci*. These may appear in chains of considerable length, are often intracellular, and usually prove on culture to be hemolytic. Streptococcal purulent effusions are not infrequently met with in young children as a sequel of broncho pneumonia, and may also occur as part of a general septicæmia or as a terminal infection in chronic general diseases. Streptococcal effusions are of less favourable prognosis than pneumococcal.

Staphylococci are occasionally found in these fluids, but they are rarely the primary cause of a local empyema. The presence of a staphylococcus is suggestive either of a general pyæmia or of an infection secondary to some other process, most commonly a tuberculous one.

Tubercle bacilli may be present in apparently purulent exudates. They will not appear in the carbol thionin preparation, but are to be suspected on the following grounds:—If the cells in the film preparation are extremely degenerate and no bacteria are to be seen; if the cultures on ordinary media are subsequently sterile; if only staphylococci or the long thin bacilli of a stinking exudate, or both are found; if the condition was a pyo pneumo thorax and not a simple empyema; if there is other evidence of tuberculosis in the patient.

Saprophytic bacilli. These are practically confined to the

stinking exudates and are usually associated with other organisms. They may be very numerous in the film preparations and appear mainly as long thin curved and often beaded extracellular bacilli. They are sometimes so numerous as to form a regular background to the film. Their diagnostic import has been already referred to. They are in all probability non pathogenic and they do not grow upon the ordinary media in aerobic culture. Sterile cultures on ordinary media are not infrequently obtained from these fluids even when streptococci and staphylococci are present in large numbers in the films.

Influenza bacilli are occasionally met with in purulent pleural exudates. They appear as minute and often intracellular bacilli and should be further investigated.

Other bacteria such as are rarely found in the chest include colon bacilli typhoid bacilli bacillus proteus bacillus pyocyaneus the pneumobacillus.

(3) Identify the causative organisms by cultural or other methods. The bacteria seen in the film preparations should suggest the further procedure necessary for their exact identification. For the majority of fluids the primary cultures should be made in litmus milk broth or dextrose broth and on blood agar slopes or plates.

In suspected tuberculous fluids proceed as follows — Shake up about 2 c.c. of the pus with about 10 c.c. of 20 per cent antiformin. Stand preferably in a warm place until the pus is mainly dissolved. Centrifuge at a high speed. Pour off the supernatant fluid and stain films of the deposit by the Ziehl Neelsen method. The bacilli may be present in such films in considerable numbers more often they are very scanty and they may not be found at all. If no bacilli are found a portion of the antiformin residue after being washed should be injected into a guinea pig and another portion cultivated upon Dorset's egg medium.

More or less clear pleural exudates. These are examined as follows —

(1) **Naked eye appearance.** The great majority of these fluids are of a pale straw colour and form an almost complete jelly like clot on standing. The exudates as a rule clot more firmly than the transudates. The presence of blood in any quantity should be noted.

(2) **The chemical examination.** This need not be at all

exhaustive and unless a considerable quantity of fluid is available can be omitted altogether. The reaction of the fluids is generally alkaline. The specific gravity is commonly above 1.020 in the case of exudates and below 1.020 for transudates. The coagulable proteins can be roughly estimated by acidifying the fluid in a test tube, boiling and allowing the precipitate to settle. The exudates usually become almost solid on boiling.

(3) The cytological examination. This is of great importance and should never be omitted. It is convenient to have two test tubes of the fluid in order that one portion may be left uncontaminated for a subsequent bacteriological investigation. If only one tube is available a portion should be poured off into a second tube for cyto-diagnosis.

The clot must first be broken up and this can be done either by shaking the tube or stirring up the contents with a sterile glass rod. The fluid is then centrifuged at a moderate speed preferably on a water or electrically driven centrifuge but a hand centrifuge will serve the purpose. All centrifuge tubes should be carefully rinsed out with distilled water before use.

After centrifuging empty out the supernatant fluid by simply turning the tube upside down. The last drop containing the majority of the cells will remain in the bottom of the tube. (It is a wise precaution to preserve the supernatant fluid if there is not a large quantity available.)

Wrap a very small piece of absorbent cotton wool around the tips of a small pair of fine pointed forceps. Soak the wool in the centrifuged deposit and make moderately thick films in the centres of two slides. Stain one slide with carbol thionin the other with Leishman's stain.

The Leishman-stained film should be treated in the same manner as a blood film but given the following times —

Leishman alone $\frac{1}{2}$ minute

Leishman + 2 volumes of distilled water 5 minutes

Distilled water only 2 minutes

Examine the films with an oil immersion lens. In the majority of these pleural fluids the cells are small lymphocytes in the minority either polymorphonuclear neutrophils or endothelial cells predominate.

The lymphocytes are often very numerous and 20 or 30 are commonly seen in one field of a $\frac{1}{2}$ th inch objective. They frequently form at least 80 per cent of the cells present the

remainder consisting of endothelials, and large lymphocytes, with occasional polymorphonuclear or other cells

Such a lymphocytic effusion is almost certain evidence of a tuberculous affection, syphilitic disease of the pleura being practically unknown. Rarely a lymphocytic exudate may occur with pleural or mediastinal neoplasms, or in the course of Hodgkin's disease

A deposit of endothelial cells occurs most frequently in cardiac or renal dropsy. They may also be found with malignant growths and are, then, frequently associated with red cells

Polymorphonuclear neutrophils may predominate in an apparently clear or in a slightly turbid fluid. They have the same significance as in an obviously purulent fluid

Eosinophil cells are occasionally found in the clear fluid which exceptionally follows a lobar pneumonia and still more rarely in a tuberculous exudate. When present in excess a hydatid cyst should always be suspected and the fluid examined for hooklets

(4) The bacteriological examination. This varies with the type of cell present

In the case of a lymphocytic exudate proceed as follows — Fill 2 centrifuge tubes (of about 10 c.c. capacity each) two thirds full of the fluid. Add one third of absolute alcohol. Invert several times and stand for 5 minutes. A copious precipitate forms. Centrifuge at a high speed for 10 minutes. Pour off the supernatant fluid. If the deposit is comparatively small in amount, make thick films from it and stain for tubercle bacilli in the ordinary way. If the deposit is very bulky (as is usually the case) fill the tubes with 20 per cent. antiformin. Shake thoroughly and stand in a warm place until the precipitate is almost dissolved. Centrifuge again at a high speed for 10 minutes. Make films of the deposit and stain for tubercle bacilli. A prolonged search is necessary to demonstrate the bacilli, and in the great majority of pleural fluids of tuberculous origin no bacilli can be found. In cases of exceptional importance the antiformin deposit should be washed two or three times with sterile saline and one portion rubbed over the surface of Dorset's egg medium the other portion injected into the leg of a guinea pig. Preferably the centrifuged deposit of the original fluid is injected, without other treatment, into the leg or peritoneal cavity of a guinea-pig. A negative result, however, does not exclude tuberculosis

Where endothelial cells are in excess no bacteriological examination is necessary

With an excess of polymorphonuclear neutrophils the procedure is the same as for purulent exudates, except that it is advisable to add the fluid in greater bulk to the media. As an alternative the fluid may be centrifuged in sterile tubes and the deposit of pus used for inoculation of the media.

Lung puncture It occasionally happens that a chest deemed to contain fluid proves on exploratory puncture to be filled with solid lung. On such occasions a small quantity of blood stained fluid is removed in the needle and should be reserved for film and culture preparations. The pneumococcus or other organisms can often be identified by these means, and a diagnosis of the condition made.

Lung puncture may be performed deliberately as a means of diagnosis but the procedure can hardly be said to be entirely free from risk and should be avoided as a routine method.

PERICARDIAL FLUIDS

Puncture of the pericardium is rarely performed. It is advised to make the puncture with a fine needle in the fourth left intercostal space half an inch from the edge of the sternum. The fluid withdrawn should be examined in exactly the same way as a pleural fluid both by cytological and bacteriological methods. Those fluids for which pericardial puncture is performed are usually purulent and frequently associated with a purulent pleurisy.

CHAPTER XIII

PERITONEAL FLUIDS—CEREBRO-SPINAL FLUIDS— SYNOVIAL FLUIDS—CYSTS ETC

PERITONEAL FLUIDS

BEFORE puncturing the peritoneal cavity the bladder must be emptied. The puncture is best made in the middle line and midway between the umbilicus and the pubes.

The fluid is examined in much the same way as a pleural fluid.

Purulent peritoneal fluids are rarely withdrawn by puncture being more commonly obtained during a laparotomy for general peritonitis. The organisms present in such fluids usually include the *bacillus coli*. Cocci are often seen in addition. Streptococci in pure culture are of the worst possible prognosis. Pure pneumococcal exudates are more frequent in young children, may be associated with comparatively slight constitutional disturbance and are of fairly good prognosis. *B. pyocyaneus* is occasionally met with and is usually associated with a particularly virulent peritonitis. Purulent fluids are sometimes obtained in association with the tubercle bacillus, a secondary infection with the *B. coli* or other intestinal organisms having taken place. Almost all of the pyogenic organisms are found from time to time in the peritoneal cavity, and the examination of purulent peritoneal fluids is conducted on exactly the same lines as those already laid down for the examination of pus generally.

Clear peritoneal fluids are examined in the same way as clear pleural fluids.

Lymphocytic fluids indicate a tuberculous lesion. endothelial fluids are associated with cardiac or renal dropsy, with cirrhosis of the liver with malignant disease or with any abdominal lesion which tends to obstruct the portal circulation.

Poly morphonuclear neutrophils may be present in apparently clear peritoneal fluids and in association with the ordinary pyogenic organisms. With such fluids it is always wise to

examine films of the deposit for tubercle bacilli otherwise the underlying cause of the condition may be missed. The tubercle bacilli may be present in considerable numbers.

The cytology of peritoneal fluids is not quite so satisfactory as that of pleural fluids. The cellular deposit is very frequently a mixed one and the diagnostic value of the findings is not very great unless the predominant cell is present in overwhelming numbers.

Lymphocytic fluids should be examined for tubercle bacilli in the same way as pleural fluids. Cultures should be made of the acute inflammatory or polymorphonuclear containing fluids.

Opalescent fluids are occasionally withdrawn from the peritoneal cavity and still more rarely from the pleural sacs. The opalescence is not removed by filtration nor by centrifuging nor by extraction with ether and is apparently due to the presence of a lipid body combined with a protein. The significance of these striking looking fluids is not known but they seem to be most frequently associated with the dropsy of parenchymatous nephritis.

Extremely rare are genuine chylous fluids in which the opalescence is due to actual fat and is removed by extraction with ether. Such fluids are associated with lesions of the thoracic duct and occur in filariasis. They are to be distinguished from the pseudochylous fluids referred to above.

CEREBRO SPINAL FLUID

The fluid is obtained by puncture through the fourth lumbar space a special hollow needle provided with a stylette being required for the operation. Needle and stylette should be made of flexible drawn nickel and of a length of 3½ inches. Do not use an ordinary steel exploring needle since it is liable to break in the tissues. An all-glass syringe attachable to the needle should be available, but it is rarely required and strong suction with it must be avoided.

The patient is placed in the left lateral position with the back well bent and the knees drawn up so as to approximate to the chin. A line is drawn across the back to join the highest points of the iliac crests and the interspinous space immediately below this line is defined with the tip of the finger. An area of skin is painted with iodine and may be

anæsthetised by eucaine or the ethyl-chloride spray. The needle is plunged firmly into the interspinous space in the middle line and pushed steadily onwards and directly forwards until the canal is reached. In the majority of adult patients the needle has to be passed almost up to the hilt and the correct position of the point is recognised by the sudden and easy passage of the needle just as the canal is reached. The patient may also complain of a sensation of pain or tingling in the leg. On withdrawing the stylette the fluid may gush out or as is more frequent escape drop by drop. The flow can sometimes be started by cautiously applying suction with the syringe.

The fluid is collected in clean sterile test tubes and if the first sample is tinged with blood a second tube should be ready as soon as the fluid runs clear. It is important to obtain a sample free from blood. Five c.c. of fluid is sufficient for diagnostic purposes.

After withdrawing the needle seal the puncture with collodion and direct that the patient be kept in bed with the head low for a period of 24 hours.

The fluid is examined as follows —

(1) **Naked eye examination.** The normal cerebro spinal fluid is a clear limpid fluid resembling water. Sometimes in pathological fluids a delicate clot having the appearance of a fine cobweb slowly forms in the tube. This formation of a clot is certain evidence of disease and suggests either tuberculous or much less commonly meningococcal meningitis. In suppurative meningitis whether due to the meningococcus or to other pyogenic organisms such as the pneumococcus every grade of turbidity is found from a mere cloudiness to actual pus. The presence of blood if bright in colour is usually due to the puncture of a vessel. If dark intimately mixed with the fluid and persisting during the whole time the fluid is running it is suggestive of intradural hæmorrhage such as occurs in fracture of the base of the skull. Rarely a clear canary yellow fluid is obtained (*xanthochromia*).

(2) **Cytological and bacteriological examinations.** If normal spinal fluid is centrifuged for 10 minutes at a moderate speed if the tube is emptied by inversion and a fairly thick film the size of a sixpence is made with a cotton wool swab in the manner described for pleural fluids practically no cells are found in the film. The presence of more than 2 or 3 cells to

a film in preparations made by this method is evidence of disease. It often happens that no obvious sediment is found after centrifuging, the last drop may nevertheless be rich in cells.

If cells are present in excess, and particularly in syphilitic or tuberculous lesions, when the numbers are rarely extreme and subject to fluctuation, the number should be estimated. The cell count may be made as follows. Shake the fluid thoroughly and make an accurate mixture of equal parts of the spinal fluid and 2 per cent acetic acid. The object of the acetic acid is to hæmolyse the red cells, which are always present in small numbers, if the fluid is grossly contaminated with blood, accurate cell counts and protein estimations are not possible. A drop of the mixture is then transferred to a Neubauer counting chamber (p. 41), and with a $\frac{1}{4}$ inch objective all the cells are counted in the 9 large squares or in 0.9 of a cubic millimetre. The number of cells counted in the diluted fluid $\times \frac{10 \times 2}{9}$ gives the number of cells per cubic millimetre of spinal fluid. This number does not exceed 5 in normal fluids.

The cells present in these fluids are practically of only two kinds—small lymphocytes and polymorphonuclear neutrophils, endothelial cells in excess are never seen. On very rare occasions in general sarcomatosis of the meninges, sarcoma cells which resemble endothelials in appearance, may be present in considerable numbers in the fluid. If small lymphocytes are present the fluid is probably either syphilitic or tuberculous, but a moderate excess of lymphocytes occurs in other conditions of which the most important are encephalitis lethargica and poliomyelitis.

If a syphilitic lesion is suspected proceed as follows. Estimate the number of cells and identify the type. Estimate the protein content and do the colloidal gold test (p. 275). Do a Wassermann reaction on the spinal fluid and with the serum. In secondary syphilis the fluid may be normal, or the Wassermann may be negative and the protein and cells slightly increased or more rarely the Wassermann reaction may be positive and the cells and protein much increased. In tabes, general paresis and meningo-vascular syphilis abnormal colloidal gold curves are present, cells and protein are increased. The Wassermann is positive in the fluid in tabes and general paresis with rare exceptions, but in meningo

vascular syphilis the reaction is negative in nearly half the cases

If a tuberculous lesion is suspected proceed as follows —

When a cobweb is present, it should either be teased out on a slide or lifted out on a cigarette paper and blotted off on to a slide, then dried and stained for tubercle bacilli. If there is no cobweb, half fill two centrifuge tubes with the fluid, and add to each an equal volume of absolute alcohol. Mix thoroughly and stand for a few minutes. In the case of normal fluids practically no turbidity results, in meningeal disease, whether tuberculous, syphilitic or suppurative, a more or less marked opalescence forms in the fluid owing to the precipitation of proteins. This stage of the proceedings therefore yields confirmatory evidence of the cytological findings. The tubes are then centrifuged at as high a speed as possible for about 10 minutes. In the majority of cases a small but obvious sediment is found at the bottom of the tubes. As thick films as possible are made from the sediment and stained for tubercle bacilli in the ordinary way. Bacilli may be present in considerable numbers, more commonly they are very scanty, a careful search however should reveal the bacilli in at least 70 per cent of the cases. If no bacilli are found 2 to 3 c.c. of the original fluid should be injected into the peritoneal cavity of a guinea-pig.

If polymorphonuclear cells are found, some form of septic meningitis is present. The causative organism should be sought for in the films. Meningococci are seen as Gram-negative diplococci, the majority of which are within the polymorphonuclear cells but a number of extracellular cocci are nearly always present as well. In meningococcal exudates the first cultures should be made on serum agar and in milk, and in a certain number of cases even when the organisms are present in the films, the cultural results are negative. Bacteria will sometimes grow out in the spinal fluid, the original cultures remaining sterile, and it is advisable to incubate a tube of the fluid with the primary culture tubes. Once a growth has been obtained the sub cultures grow readily, provided they are made at frequent intervals. Pneumococci are sometimes found in the spinal fluid, and are recognised as Gram positive, lanceolate, extracellular diplococci. Pus cells may be scanty and the fluid milky in appearance owing to the large numbers of bacteria present. The pneumococcal cases run a rapidly

fatal course of 1 to 3 days as a rule consequently the prognosis is far less favourable than in the meningococcal infections which run a more chronic course with a fairly high percentage of recoveries. Other organisms which may be found in the cerebro spinal fluid include streptococci influenza bacilli and colon bacilli. Diphtheroid bacilli and staphylococci are occasionally grown in culture but are usually to be regarded as skin contaminations. Streptococcal or pneumococcal meningitis is always secondary to a focus elsewhere and usually to suppuration in the middle ear. Influenzal meningitis is most frequent in young children and usually runs a rapid and fatal course. The bacilli in the fluid are commonly large and curved.

To recapitulate the findings of a spinal fluid deposit — Absence of cells is very strong evidence against any variety of meningitis. The normal fluid contains less than 5 cells to the cubic millimetre. Small lymphocytes suggest syphilis or tuberculosis. Polymorphonuclears mean septic meningitis of which the most common variety is due to the meningococcus the next common to the streptococcus or pneumococcus.

The examination of the cellular deposit may be obscured by the presence of blood and it must also be recognised that the preponderance of the cells whether lymphocytes or polymorphonuclears is a matter of degree. A few polymorphonuclears will be found in a tuberculous or syphilitic meningitis. Lymphocytes are fairly numerous in meningococcal infections and particularly in the more chronic cases. A positive interpretation of the film requires that the percentage of the predominant cell should be at least 70.

(3) The chemical examination. *The protein content of the fluid is always examined and a rough guide for ordinary clinical purposes is afforded by the alcohol test.* About 2 c.c. of the fluid is poured into a test tube and an equal amount of absolute alcohol is carefully run down the side of the tube without mixing the fluids. With a normal protein content the line of junction of fluid and alcohol is only just visible. If protein is present in excess a turbid ring develops at the line of junction of the two fluids. The alcohol and spinal fluids are then mixed and the appearances of the ring test confirmed from the resulting general opacity. With a normal fluid a very faint opalescence results. With increasing amounts of proteins the reading varies from a definite turbidity to a

course flocculent precipitate. The protein content is increased in all forms of meningitis and the increase usually corresponds roughly with the number of cells present. In cases of spinal or cerebral tumour or with meningeal adhesions the protein content is high and there is no cellular increase. The yellow spinal fluids referred to previously are of this nature and have a very large excess of protein. The condition is known as From's syndrome or xanthochromia.

Protein estimation

Required (1) 0.3 per cent solution of serum proteins. This can be made up sufficiently accurately from normal serum by assuming that it contains 7 per cent of protein and diluting accordingly with normal saline.

(2) Five per cent solution of sulphosalicylic acid.

(3) Graduated 1 c.c. and 10 c.c. pipettes.

(4) Series of test tubes of clear glass and equal bore.

Procedure To a series of tubes add 0.1, 0.2, 0.3, 0.4, 0.6 and 1.0 c.c. of the protein solution and normal saline to make the volume up to 3 c.c. Add 3 c.c. of sulphosalicylic acid solution to each and mix. Take 0.5 c.c. of the cerebrospinal fluid in another tube, add 0.5 c.c. of saline and 1 c.c. of sulphosalicylic acid solution and mix. Compare this tube with the tubes containing the standard protein solution. From the tube which it most nearly matches in degree of opacity the protein content of the cerebrospinal fluid can be calculated. The tubes correspond to concentrations of 0.02, 0.04, 0.06, 0.08, 0.12 and 0.20 per cent of protein.

PROTEIN IN CEREBROSPINAL FLUID

Normal	0.020 to 0.035 per cent
Meningitis	0.050 to 0.20
Disseminated sclerosis	0.030 to 0.060
General paralysis	0.040 to 0.10
Tabs	0.030 to 0.060
Syphilitic meningitis	0.050 to 0.20
Xanthochromia	up to 2

Lange's colloidal gold test is of considerable diagnostic value in syphilitic infection. It is not merely confirmatory of other tests but is of particular assistance in the diagnosis of the early stages of tabs and general paresis. In the latter affection we have on several occasions found a positive colloidal gold curve.

at a time when all other tests were negative. The reaction depends upon the altered globulin albumin ratio in the spinal fluid by which the protective action of the albumin in preventing the metallic gold precipitation by the electrolyte or salt is overcome by the precipitating action of the globulin. The test is performed as follows —

Required (1) Special distilled water. This is most conveniently made from tap water to which a knife point of potassium permanganate has been added. This is distilled using a distilling flask and condenser without rubber stoppers or connections. The first portion of the distillate should be rejected.

(2) Hard glass test tubes

(3) Graduated 1 c c pipette 2 graduated 10 c c pipettes

(4) Colloidal gold sol. To 200 c c of special distilled water in an Erlenmeyer flask add 2 c c of a 1 per cent solution of pure potassium oxalate. Heat to boiling and then run in drop by drop 2 c c of a 1 per cent solution of gold chloride (double salt $\text{AuCl}_3 \cdot \text{NaCl} \cdot 2\text{H}_2\text{O}$). After a few seconds a brilliant red colour develops. Continue boiling until all the gold solution has run in and cool. The sol should be bright red with no suggestion of blue or purple clear by transmitted light and slightly cloudy by reflected light. Difficulties in the preparation arise mainly from the use of acid solutions of gold chloride. If the solution is not too acid it may be made serviceable by neutralisation.

(a) 0.4 per cent sodium chloride solution made with special distilled water

All glass ware must be cleaned by soaking in sulphuric acid bichromate mixture and washed out well with tap water followed by special distilled water.

Procedure Place 10 of the test tubes in a rack. To the first add 0.9 c c of the sodium chloride solution and 0.5 c c to the remainder. Add 0.1 c c of the spinal fluid to the first tube mix. Remove 0.5 c c of this mixture and add to the second tube mix. Add 0.5 c c of this mixture to the third tube and so on with the subsequent tubes discarding the last 0.5 c c. Add 2.5 c c of the colloidal gold to each tube. Mix and let stand overnight. Complete precipitation with a colourless supernatant fluid is reported as 5 a greyish blue fluid as 4 a blue fluid as 3 reddish blue as 2 bluish red as 1 and unchanged as 0.

In general paresis complete precipitation (5) is found in the earlier tubes, in tabes changes up to 4 or 5, with the maximum usually about the fourth tube, in disseminated sclerosis changes up to 3 or 4, with a maximum about the fifth tube. In tuberculous and acute forms of meningitis the maximum changes (2 to 3) occur in the higher tubes. Normal fluids may change to 1 or even 2, usually about tube 8.

The glucose in cerebrospinal fluid normally lies between 0.06 and 0.09 per cent. Low concentrations are found in hypoglycæmia produced by insulin, also in acute, and particularly in tuberculous meningitis (0.01 to 0.05 per cent).

The method of estimation described for blood is applicable to cerebrospinal fluid. Much smaller quantities can be used, it is convenient to measure 0.5 c.c. of cerebrospinal fluid into a 5 c.c. measuring cylinder, add water to about 3.5 c.c., then 0.5 c.c. of sodium tungstate and 0.5 c.c. of $\frac{2}{3}$ N sulphuric acid, make up to 5 c.c., and proceed as for blood.

The chloride in the cerebrospinal fluid normally lies between 0.43 and 0.45 per cent, reckoned as chlorine (0.71 and 0.75 reckoned as sodium chloride). Low figures may be found associated with low plasma chloride, for example in chronic nephritis (though not invariably) and in acute intestinal obstruction, also in acute, and particularly tuberculous, meningitis, in which the concentration may fall as low as 0.58 (reckoned as sodium chloride). This reduction in meningitis may be of value in diagnosis. High values may be found in cases of nephritis with nitrogen retention.

Chlorides may be estimated in cerebrospinal fluid by the method described for plasma.

SYNOVIAL FLUIDS

The puncture of joints for diagnostic purposes should only be undertaken in ideal surroundings and with the strictest aseptic precautions. Provided there is a large excess of fluid in one of the large joints and every care is taken, there is no real risk in the procedure. The point at which to make the puncture necessarily varies for each joint, but no difficulty should be experienced if the needle is plunged directly through the skin into the most obviously distended portion of the joint.

cavity The needle should be of sharp steel fitted with an all glass syringe and with a stilette After withdrawal of the needle the puncture wound must be sealed with collodion

The removal of the fluid from a joint has further advantages The relief of tension when a moderate quantity of fluid is withdrawn is in many cases beneficial and the subsequent preparation of an autogenous vaccine may be made use of as an aid to treatment

In the puncture of a distended knee joint an unexpected small quantity of fluid is commonly withdrawn owing to the blocking of the needle with synovial fringes By altering the position of the needle a further flow is often obtained

The normal synovial fluid is a sticky viscous material of unmistakable character In disease it may be not obviously altered in appearance or it may be turbid, or it may be obviously purulent

In all cases film preparations should be made without centrifuging In practically every variety of arthritic disease the predominant cell is a polymorphonuclear neutrophil while lymphocytic and endothelial cells are usually present in addition The polymorphonuclear cell is predominant not only in acute lesions due to gonococci or other pyogenic organisms, but also in the chronic distended joints of rheumatoid arthritis Lymphocytes may be more numerous in acute tuberculous joints but the cytology of synovial fluids is not of striking diagnostic value The cells in carbol thionin preparations are seen lying on a deeply stained background of the synovial fluid

All film preparations should be carefully searched for bacteria and these should be seen in all the acute septic cases due to streptococci pneumococci, or staphylococci and in many of the gonorrhoeal cases if a very careful and prolonged search is made In these affections the findings of the film preparations must always be confirmed by cultural methods

In tuberculous cases no organisms are seen in the carbol thionin films unless a secondary infection has taken place The material withdrawn in such cases is often suggestively caseous and the cells are necrotic and many of them unrecognisable A portion of the fluid should be treated with antiformin, centrifuged and examined for tubercle bacilli in the ordinary way The bacilli may be fairly numerous

In the large group of arthritic affections known as rheumatoid no organisms are seen in the films and none is grown in any culture media. Paracentesis of acute rheumatic joints is rarely performed partly because the effusion is so often slight in amount and is mainly periarticular.

Hydrocele and spermatocele fluids These are obtained by puncture through the tensest portion of the cysts care being taken to avoid wounding one of the scrotal vessels. Hydrocele fluid is commonly clear and straw coloured. On standing a quantity of cholesterol crystals separates out. The cells present are mainly endothelial and no organisms are found. In cases where secondary infection has occurred polymorphonuclear neutrophils predominate and the ordinary pyogenic organisms most commonly staphylococci are obtained. Spermatocele fluid is usually milky in appearance and in almost all cases large numbers of spermatozoa are seen under the microscope. The spermatozoa are best examined unstained a drop of the fluid being placed on a slide with a cover slip over it and watched under the $\frac{1}{8}$ inch objective. Stained preparations of dried films can be made with carbol thionin or Leishman's stain.

CYSTS

For all cyst fluids a chemical and a microscopical examination are required. Bacteriological investigations are rarely called for. In the majority of cases the nature of the cyst is clearly determined on clinical grounds or at operation and the laboratory investigations are for purposes of confirmation. The examinations made should naturally be those most appropriate to the type of cyst suspected. When the nature of the cyst is quite obscure the following routine procedure should be adopted. Note the amount and naked eye appearance of the fluid. Test the reaction and the specific gravity. Estimate roughly the amount of coagulable protein. Test for the presence and amount of urea. Test for the presence of a reducing substance. Estimate roughly the amount of chlorides present. Examine the centrifuged deposit in fresh and stained specimens as to the nature of the cells and the presence of crystals or of hydatid hooklets. If there is reason to suspect a pancreatic cyst examine also for the presence of ferments. Prepare paraffin sections of the cyst wall if available.

The following is a brief description of the more important cyst fluids —

Hydatid Cysts

Specific gravity about 1,010
 Proteins very scanty or absent
 Chlorides abundant
 Sugar occasionally present
 Urea present in very small amount
 Hydatid hooklets in centrifuged deposit

The really diagnostic feature is the presence of hooklets. The hooklets are sharply pointed at one end and barbed. Occasionally a well formed scolex with a circle of hooklets may be found (p 158, Fig 14)

Pancreatic Cysts

Specific gravity, 1 010—1,020
 Reaction alkaline
 Proteins in varying amounts
 Chlorides usually scanty
 Urea often a trace
 Cholesterol usually present
 Trypsin, lipase, and diastase present

The characteristic constituents of these cysts are the ferments which can be found in the majority of cases. They are tested for as follows —

Trypsin

Required Casein solution. Dissolve 0.1 gram of casein in 100 c c of 0.1 per cent Na_2CO_3 .

In 4 test tubes place 10 c c of the casein solution warmed to 37° C. Add 0.1, 0.2, 0.5 and 1 c c of the fluid to be tested. Incubate at 37° C for 15 minutes. To each add a few drops of 1 in 40 acetic acid. If the casein is digested, the tube will remain clear. If x was the minimum amount of the fluid tested required to effect digestion the 'tryptic activity'

$\frac{1}{15x}$

Trypsinogen may be tested for if trypsin is not found. Prepare enterokinase by grinding 1 gram of fresh fat free duodenal mucosa of a pig with sand. Add 10 c c of normal

saline and strain through muslin. Add 1 c c of this extract to 2 c c. of the cyst fluid, incubate at 37° C for 30 minutes. Then test for trypsin as before.

Lipase

Boil 5 c c of milk to kill bacteria. Cool and add 2 c c of the fluid. Add 1 c c of 0.02 per cent phenol sulphone phthalein and enough 2 per cent sodium carbonate to give the solution a reddish tinge. Divide into two portions, A and B. Boil B and cool. Incubate at 37° C. If lipase is present A will become yellow owing to the setting free of fatty acids. Tube B serves as a control.

Diastase

Dilute 1 c c with 9 c c of normal saline and test for diastase as described in Chapter XVI.

Renal cysts. Renal cysts proper may arise from a hypernephroma, from a congenital cystic kidney, or from a contracted granular or cardiac kidney. Rare single cysts of considerable size may be found arising, as a rule, from the lower pole of an apparently healthy kidney. The composition of such cysts is very variable and both urea and uric acid may be absent.

The contents of a hydronephrosis have more obviously the composition of urine, but the specific gravity is usually below 1.010, albumin is frequently small in amount. The reaction may be acid, a point which immediately suggests a renal origin. The most important constituent is urea, which may amount to from 0.4 to 1.0 per cent. It must be remembered, however, that many cystic fluids contain a trace of urea, though a percentage of more than 0.2 is very suggestive of a renal origin and more than 0.5 is practically diagnostic.

Ovarian Cysts. The source of the fluid may sometimes be recognised from the character of the cellular deposit. The cells in some cases may be indistinguishable from the endothelial cells of a peritoneal fluid, in other cases columnar epithelial, or even ciliated, cells may be found. Cholesterol crystals are common in these fluids, but may occur in almost any variety of cyst.

Other abdominal cysts. These include mesenteric, retroperitoneal, and omental cysts. All of these are rare, but they may be of considerable size. The fluid contents present no particularly characteristic feature.

SECTION IV—THE URINARY SYSTEM

CHAPTER XIV

ROUTINE EXAMINATION OF THE URINE

AN examination of the urine forms a part of the routine investigation of every patient. For the purposes of an ordinary clinical examination a specimen of urine passed at the time can be made use of but in the case of patients confined to bed a sample taken from the total urine passed in the 24 hours is to be preferred. For examination for casts for glucose in patients under treatment with insulin and for detection of fixation of specific gravity the first specimen passed in the morning should be examined. For special purposes the urine must be withdrawn by catheter and a catheter specimen is necessary for bacteriological investigation in the case of women. For the detection of small traces of albumin or of pus in the urine of women a catheter specimen is similarly essential since a sample of urine passed in the natural manner is very frequently contaminated by leucocytes and the albuminous secretion of the vagina. In special cases the secretions of each kidney should be obtained by means of ureteric catheterisation. The routine examination of an ordinary specimen of urine should always be conducted according to a settled scheme of investigation. The simple tests described below must be applied in every case whether there is any reason to suspect any abnormal substance in the urine or not. The urine may be found loaded with albumin or sugar in the case of apparently healthy persons who are seeking advice as to the necessity of some operation that may well be postponed but which would be advisable for normal persons. The unexpected detection of pus in the urine may alter the diagnosis of a supposed splenic enlargement into that of a left pyonephrosis and numerous similar instances of the importance of routine examination could readily be adduced.

Amount The amount of urine passed in 24 hours by a

normal person varies considerably, particularly with the amount of fluid drunk and lost by the skin, the figure 1,500 c c is only an average and subject to wide variation. When conditions are to some extent standardised, as with patients in hospital, the variations may be less.

Apart from variations due to intake and loss by the skin, a greatly increased volume of urine is passed by patients with diabetes mellitus who are excreting large quantities of glucose, and is the essential abnormality in diabetes insipidus. Patients who are recovering from œdema may pass very large quantities of urine. Large volumes are said to be passed by patients with chronic interstitial nephritis, when they are taking a diet containing a normal amount of protein the volume of urine is moderately increased in order to get rid of the urea formed, but on restricted diets in hospital they rarely pass more than 2,000 c c in the 24 hours, the volume passed at night however is larger than is usual with normal persons.

Decrease in the volume of urine occurs in any condition in which the intake of water is reduced or the loss by sweating is increased, as in fevers, also, as the result of loss of fluid by diarrhœa and vomiting. It also results from failure of excretion in heart failure, acute nephritis, nephrosis and in the terminal stage of other forms of nephritis. Complete suppression of urine results from blocking of both ureters by calculi, and may follow operations on the genito urinary tract.

Decrease in the urinary output must be distinguished from retention of urine, such as commonly takes place with stricture of the urethra or in obstruction due to enlargement of the prostate, and may also occur in certain cerebral conditions, such as meningitis, and after operations.

Appearance Normal urine, when passed, is usually clear, but occasionally turbid from the presence of phosphates, it commonly becomes turbid on standing owing to the deposition of urates. Turbidity in the urine of patients when passed may be due to phosphates, pus, bacteria, or, very rarely, fat.

Colour The yellow colour of urine is due to urochrome, the main cause of variation of colour is the volume passed, large volumes being pale, and small dark, but some variation is also caused by the diet. An abundance of green food produces a darker urine.

The most important causes of dark urine are blood pigments and bile pigments. The abnormal colours produced in urine

by urobilin, hæmatoporphyrin, homogentisic acid, phenol derivatives, melanin, eosin, methylene blue, and pyramidon will be considered later as the recognition of these substances cannot be included in routine examination of urine

The reaction The reaction of freshly passed normal urine is usually acid but may be alkaline when the diet consists mainly of vegetables and after taking alkalis. The fresh urine of patients may be alkaline from the same causes, or owing to bacterial action in the bladder. Unless some preservative is added, or the urine collected with aseptic precautions, the urine becomes alkaline on standing owing to the breakdown of urea so that no significance can be attached to an alkaline reaction in urine taken and kept without precautions.

For routine examination it is sufficient to test the reaction with litmus paper, care should be taken that the urine does actually soak into the paper, especially if this is glazed.

Specific gravity The specific gravity of the urine of normal persons varies considerably, depending mainly on the volume and the amount of urea excreted. The specific gravity of the night urine is usually higher than that of the day urine.

High specific gravity, that is above 1.020, is usually found in all conditions in which the volume is low, but occurs, associated with a large volume, in diabetes mellitus when much glucose is excreted. Low specific gravity again is usually associated with a large volume, in nephritis with severe impairment of renal function the specific gravity is constantly low and about 1.010.

The presence of albumin Albumin in quantity detectable by the ordinary tests is normally absent from the urine and its presence in even small amount is of important clinical significance. The albumin present in disease consists in the great majority of cases mainly of serum albumin with varying amounts of serum globulin. One of the two following tests should be employed in every examination, and either test if properly performed is perfectly reliable —

(a) *The heat test* The urine, if cloudy, must be filtered, and if alkaline must be rendered acid to litmus with dilute acetic acid (1 in 40), the 33 per cent solution frequently used is much too strong. Fill a clean test tube two thirds full with the clear acid urine. Slowly heat to boiling point over a small Bunsen flame the top inch of the fluid, rotating the tube but

not shaking it. Look at the column of fluid with the light coming from behind and holding the tube against a dark background. If a cloud, however faint, appears in the heated portion, add a few drops of the dilute acetic acid, whether the urine was previously acid or not. If the cloud persists, albumin is present, if the turbidity vanishes again, it was due to phosphates. If the urine remains clear after heating, add a little acetic acid, and heat again, a turbidity due to albumin is sometimes found. If the urine remains clear, albumin is absent. The failure to examine the tube against a dark background is responsible for innumerable cases in which a small, but often important, trace of albumin has been altogether missed. Unless a considerable cloud of coagulable protein is present the turbidity becomes transparent when held against a bright or comparatively bright ground. Even should the urine be slightly turbid with bacteria (gross turbidity from this cause can be modified by centrifuging at a high speed), the additional cloud of albumin can be detected in this way.

If the urine is turbid from the presence of phosphates, the turbidity will disappear on adding the acetic acid.

If the turbidity is due to the presence of urates and is not completely removed by filtration, gently warm the upper half of the tube, which will become clear before proceeding to heat the top half inch of the fluid. If such a urine is heated rapidly, the turbidity of the albumin may merely replace that of the urates and escape observation.

Very rarely in very dilute urines albumin does not precipitate on boiling owing to the absence of salt, if this is suspected 1 c c of 2 per cent sodium acetate should be added to about 10 c c of urine, followed by sufficient acetic acid to make the urine definitely acid, and the urine boiled as before.

(b) *The sulphosalicylic acid test* This test is simple and trustworthy. Take 2 inches of urine in a test tube, if alkaline, make slightly acid with acetic acid, and add 10 drops of 20 per cent sulphosalicylic acid. If albumin is present a cloud appears.

The nitric acid test (Heller's test) This test, though consecrated by tradition, cannot be recommended. It is not very delicate, and is given by several substances other than coagulable proteins.

In routine examination, when albumin is found in urine, it is

advisable to make a rough estimate of the amount present as follows. If a cloud only appears such as would cause no appreciable bulk of precipitate at the bottom of the test tube note that a faint trace a trace or a heavy cloud of albumin is present according to the opacity which is present after boiling and acidifying. If a precipitate is formed in bulk boil the whole contents of the test tube and allow to stand. When the precipitate has all collected at the bottom hold an ordinary tape measure against the test tube and read the level of the precipitate and the urine. Express the depth of the precipitate as a fraction of the depth of the column of urine as one sixth one fourth one third etc.*

The following are among the more important conditions in which albumin occurs in the urine —

Albumin may occur after violent exercise severe exposure and after excessive eating or drinking. In the previously normal individual the albuminuria which may be induced by such means rapidly disappears. Fever particularly if considerable or prolonged is commonly associated with a trace of albumin.

Large amounts of albumin (one fifth or more) are present in the urine in acute nephritis nephrosis and nephrosis mixed with glomerulo nephritis in the kidney lesions associated with pregnancy and usually in heart failure when the volume of urine is low. In chronic glomerulo nephritis (chronic interstitial nephritis) moderate amounts are found (one twelfth to one fifth) the statement that only traces might be present in chronic interstitial nephritis arose from the confusion between nephritis and hyperpiesia.

A trace of albumin is often found in the urine in diabetes mellitus.

In cases of albuminuria due to any form of renal disease casts are nearly always present in addition.

Albuminuria may also occur and in considerable degree without any known structural alteration in the kidneys or heart. Such cases are known as functional or postural albuminuria and are not uncommon in young people. Such albuminuria tends to disappear when the patient is at rest and to reappear on taking exercise or even on assuming the erect posture. Casts are absent and the arteries are not affected.

* If the fraction is one half the urine contains about 1 per cent of albumin if one-eighth about 0.2 per cent and if a cloud only about 0.0 per cent.

nor do these cases commonly appear to progress to any of the recognised forms of nephritis

The occurrence of blood or pus in the urine is always associated with the presence of albumin and it is frequently of importance to determine whether the amount of albumin is such as could be accounted for by the quantity of pus or blood or is in excess of it. Albumin present in greater amount than can be accounted for by the amount of pus coming from a probable focus in the bladder for example would point to involvement of the kidneys as well. It may be taken as a rough guide that a very considerable amount of pus in the urine produces little more than a trace of albumin. The presence of blood leads to a higher degree of albuminuria than the same amount of pus and hæmaturia occurring with a chronic interstitial nephritis obscures the albumin due to the nephritis. In such cases the presence of granular casts would indicate renal involvement. The hæmaturia which accompanies acute nephritis is usually associated with a degree of albuminuria obviously much in excess of that accounted for by the amount of blood present.

The exact significance to be attached to the presence of albumin in the urine is of great importance in life insurance and every case in which even a trace is discovered calls for a very complete examination not only of the urine but of the patient also. There is no doubt that transient albuminuria may occur without any serious involvement of the kidney and in exceptional cases a considerable percentage of albumin may be passed over long periods without any apparent detriment to the individual.

Reducing substances In this examination two questions arise (a) Is a reducing substance present? and (b) if a reducing substance is present what is it?

(a) For the detection of the presence of reducing substance two methods Fehling's and Benedict's can be recommended. It is advisable to become familiar with one of these methods and use it only.

Fehling's method Preparation of solution —

(1) Dissolve 34.64 grams of powdered crystalline copper sulphate in warm water cool and make up to 500 c c

(2) Dissolve 180 grams of sodium potassium tartrate in 300 c c of hot water filter add 70 grams of pure caustic soda, cool and make up to 500 c c

For use, mix equal volumes of (1) and (2)

To test urine,* take an inch of urine in one test tube and an equal volume of Fehling's solution in another. Boil both, add the Fehling's solution to the urine, and mix well. Allow the mixture to stand till cool before deciding that the reaction is negative. With abundant reducing substance, *e.g.*, over 1 per cent of glucose, a reddish or yellow precipitate of cuprous oxide appears at once, while if the amount of reducing substance is small, *e.g.*, under 0.5 per cent of glucose, nothing but a greenish deposit may appear on standing, with some urines containing glucose and a high concentration of other substances as well, the cuprous oxide is further reduced to copper in colloidal solution giving a red or purple fluid with no precipitate.

It is necessary to boil the Fehling's solution before adding to the urine as the mixed solution may change on keeping so that it reduces itself on boiling, also glucose is partly destroyed if boiled with this strongly alkaline solution, so that the test is less delicate.

This test will detect about 0.2 per cent of glucose in urine so that it is sufficiently delicate for clinical use. A very rough idea of the amount present may be obtained by dropping hot urine with a pipette into 1 inch of Fehling's solution in a test tube which is kept boiling during the addition. If 10 drops are required 100 c.c. of the urine contain about 2 grams of glucose.

Benedict's test Preparation of solution —

(1) Dissolve 173 grams of sodium citrate and 100 grams of anhydrous sodium carbonate in 600 c.c. of water, filter into a litre cylinder and dilute to 850 c.c.

(2) Dissolve 17.3 grams powdered crystalline copper sulphate in 100 c.c. of water and dilute to 150 c.c., add this solution to the citrate-carbonate solution in a beaker, with constant stirring.

To 5 c.c. of this solution, in a wide test tube add 10 drops of the urine, free of albumin, to be tested, boil for 2 minutes and allow to cool. If a reducing substance is present a precipitate will appear, depending, as with Fehling's solution on the amount of this substance. This test is more delicate than Fehling's.

(b) If a reducing substance is found in urine and the evidence

* If albumin is present in the urine, coagulate this by boiling as already described, filter, and test the filtrate.

derived from clinical examination and estimation of the blood sugar does not unquestionably point to the diagnosis of diabetes mellitus it is necessary to investigate whether the reducing substance is glucose or one of the other reducing substances which may occur in urine. As this investigation is not part of a routine investigation of urine it will be considered later (Chapter XVI p 312)

Acetone bodies are excreted when the metabolism of carbohydrate is much reduced either from inability to burn glucose as in diabetes mellitus or from absence of carbohydrate to burn as in starvation. They are also excreted in other conditions particularly in children with fever and in pregnancy amounts sufficient to give a moderate nitroprusside reaction may be found in the urine of pregnant women who otherwise appear perfectly normal. Larger amounts may be found associated with abnormal nausea and vomiting.

Acetone bodies are formed from the incomplete breakdown of fats and some amino acids. When the amount formed is small acetone ($\text{CH}_3 \text{ CO CH}_3$) is mainly excreted together with a little aceto acetic acid ($\text{CH}_3 \text{ CO CH}_2 \text{ COOH}$). As the amount excreted increases β hydroxybutyric acid ($\text{CH}_3 \text{ CHOH CH}_2 \text{ COOH}$) appears in increasing quantities and the amount of aceto acetic acid is increased to a less degree.

Tests for these substances should be made regularly in all cases of diabetes mellitus and it would be as well if the tests were made more frequently in other conditions as more information as to the morbid states in which these substances occur would be valuable.

Rothera's test should always be used before any other, as it is more delicate than the iodoform test and not given by other substances which may occur in fresh urine, as is the ferric chloride reaction. Take solid ammonium sulphate in a test tube to the depth of about 2 inches fill the test tube about two thirds full of urine shake well to saturate the urine with ammonium sulphate add 3 drops of a strong, freshly prepared solution of sodium nitroprusside and $\frac{1}{2}$ inch of strong ammonia solution mixing after each. If acetone or aceto acetic acid is present a permanganate colour develops, reaching its maximum in about 15 minutes. Neither urine nor nitroprusside solution should be heated. This test is given by both acetone and aceto acetic acid it will detect them in urine when over 0.005 per cent of the two substances are present.

The use of fresh nitroprusside solution daily is wasteful, a permanent reagent may be made as follows. Dissolve 71 grams of ammonium nitrate and 5 grams of sodium nitroprusside in water and make up to 200 c c, keep in an amber bottle in the dark. For use add 1 c c of this solution to 3 c c of urine, mix and add about 0.5 c c of strong ammonia solution. The colour resulting is less intense and develops more slowly than that produced in the usual way. The reagent will keep several months.

For acetone alone the *iodoform reaction* may be used. To 2 inches of urine in a test tube add about $\frac{1}{4}$ inch of strong caustic soda, filter if cloudy, and to the filtrate add 1 c c of Gram's iodine. If acetone is present the smell of iodoform may be detected or iodoform may precipitate. This is a very delicate test for acetone in water but in urine the smell may be obscured by other smells and the precipitate held up by colloids, so that the test is not very satisfactory.

For the detection of acetoacetic acid the *ferric chloride reaction* is commonly used. To 1 inch of urine in a test tube add ferric chloride (10 per cent) solution drop by drop, a precipitate usually forms which clears up on adding more ferric chloride solution. If acetoacetic acid is present in quantities over 0.02 per cent a brownish-red colour appears. A similar colour is given by products of phenol, particularly salicylic acid and its derivatives*, also by derivatives of rhubarb and senna which are excreted in the urine. The test had become established before the present popularity of aspirin and its use persists although far more than half the 'ferric chloride reactions' must be due to substances other than acetoacetic acid. It is true that the reaction due to acetoacetic acid can be distinguished. If the urine is boiled sufficiently long the acetoacetic acid is broken down so that the reaction with ferric chloride is no longer given. But the boiling must be more prolonged than that usually given. Boiling after adding ferric chloride destroys the colours due both to acetoacetic acid and to other substances. Salicylic acid derivatives when present in small quantities cannot be distinguished by the colour alone, this may exactly resemble that given by acetoacetic acid. For these reasons Rothera's test should always be

* Salicylic acid and derivatives may also give rise to a reducing substance in urine thus producing a very successful trap—a urine containing a reducing substance and giving a ferric chloride reaction but having nothing to do with diabetes mellitus.

used before trying the ferric chloride reaction if Rothera's test is negative any ferric chloride reaction found is not due to aceto acetic acid

Unfortunately there is no simple test for β hydroxybutyric acid

The condition in which acetone bodies appear in the urine is called ketosis owing to an unfortunate confusion of ideas the term acidosis has been and still is used for this condition This leads to difficulties as acidosis may occur without any appreciable amount of acetone bodies in blood or urine and a ketosis may actually be caused by alkalosis

Bile pigments occur in the urine when excessive amounts are present in the blood their detection in the urine gives little information as they are not excreted with slight degrees of jaundice and sometimes not when the jaundice is moderately severe Their presence in the urine is as a rule fairly obvious to the naked eye and if a test tube containing urine is shaken a greenish yellow froth appears on top

The following tests may be used —

Gmelin's test Filter several cubic centimetres of urine through a clean filter paper When all the urine has passed through dip a glass rod in yellow nitric acid and then on the filter paper

A spreading ring of colours appears round the area touched by the acid The colours are from within outwards yellow red violet and green The test is not positive unless the green colour is certainly seen

Should the bile pigments be present in small amount a positive reaction may be obtained if a considerable bulk of urine is filtered two or three times through the paper

Iodine test Take 2 c c of urine in a test tube

Carefully pour on the top of the urine 1 c c of tincture of iodine

A green ring appears at the junction of the liquids

Bile salts are usually excreted only during the early days of obstructive jaundice Apparently the bile salts which usually circulate from the liver to the intestine by the bile passages and back through the portal vein are lost through the kidney in this condition and if any more are formed the amounts that are excreted are too small to be detected

The test usually employed is *Hay's test* Sprinkle a little flowers of sulphur on the surface of the urine If bile salts are

present the sulphur sinks, owing to the reduction of the surface tension of the urine, otherwise the sulphur floats

The presence or absence of bile salts seems to be of little clinical significance

Blood in the urine may be in such amount as to colour the urine bright red, and specimens are not infrequently met with which appear to contain rather more blood than urine. Blood in lesser amount darkens the urine and gives it a peculiar "smoky" appearance. Blood in small traces may be present without any alteration in the appearance of the urine

During menstruation the examination of the urine should be avoided owing to its frequent contamination by blood. Blood may be found in the urine in numerous conditions

The blood may come from any part of the urinary tract—from a chancre of the penis, from the urethra after injury, from the prostatic plexus of veins in enlargement of the prostate, from the bladder in cases of vesical calculus, tuberculosis or new growths from the ureter during the passage of a stone or from the kidneys in cases of nephritis both in its acute form and less commonly in its chronic form, in eclampsia, in calculus, tuberculosis, neoplasm, or in paroxysmal hæmoglobinuria. Among less common causes of hæmaturia are hydronephrosis, congenital cystic kidneys, kinking of the kidney over an abnormal renal artery, injuries to the kidney, and acute infections of the urinary tract. The exact site of the hæmorrhage can usually be determined by an examination of the history and physical signs of the patient together with a further examination of the urine, and in cases of difficulty by a cystoscopic examination. A further clue to the origin of the blood may be obtained by observing the relation of the blood to the urine in a sample as it is passed. If the majority of the blood is passed with the first portion of urine, the hæmorrhage is probably from the urethra or prostate. If the majority is passed at the end of micturition, it probably comes from the bladder. If blood and urine are intimately mixed throughout, the bleeding may be of renal origin.

In addition to the presence of actual blood in the urine, the same coloration may be due to hæmoglobin only. The hæmoglobin may be either oxyhæmoglobin or methæmoglobin. Hæmoglobinuria occurs in black water fever, and recurrent paroxysmal attacks of hæmoglobinuria may take place in

apparently healthy individuals in this country, particularly after exposure to cold (p 184)

For red blood corpuscles and hæmoglobin the guaiac reaction or a similar test is commonly used

The guaiac test Boil 1 inch of urine in a test tube Allow to cool

Add 2 or 3 drops of tincture of guaiacum A white precipitate forms Pour gently down the side of the tube 1 mch of ozonic ether Allow to stand

A blue ring appears at the junction of urine and ether if blood is present

This test is a fairly delicate one for the presence of blood or hæmoglobin It is given also by the urine of a patient who is taking iodides In the presence of pus in any quantity a greenish blue ring is given Since the test is not infallible, and may be given by either blood or hæmoglobin and may not be given in the presence of small quantities of blood *the urine should always be examined microscopically* as described under "deposits"

If insufficient red corpuscles are found, to account for the colour of the urine or the depth of the guaiac reaction, it is necessary to examine the urine spectroscopically to make sure that the pigment is hæmoglobin or methæmoglobin A small direct vision spectroscope can be used and the urine, if deeply tinged, should be diluted with water When methæmoglobin is present the band of methæmoglobin is seen in the red, the bands of oxyhæmoglobin are usually present also If the band in the red alone is present add some solid sodium hydrosulphite and shake, the band is replaced by the band of reduced hæmoglobin

CHAPTER XV

ROUTINE EXAMINATION (*continued*)—DEPOSITS

ORGANISED URINARY DEPOSITS

THE examination of urinary deposits with the microscope is more important and more neglected than almost any other laboratory investigation. The student working in the wards is more concerned and rightly, with the physical signs and symptoms of the patient, consequently he prefers to content himself with a note of the naked-eye appearance of a deposit or at the most with a rough chemical test. The recognition of the various formed elements which may be present in the urine requires some practice, and this is more conveniently acquired in a laboratory than in the wards. The time available in the wards is limited, and the ward microscope, however excellent an instrument it may have been in its youth is apt to reflect more light from its brass work than it admits through its lenses. Fortunately for those students and practitioners who have been unable to avail themselves of a laboratory course an acquaintance with urinary deposits is fairly readily acquired without personal instruction. The deposits particularly lend themselves to accurate reproduction by diagram and are among the minority of pathological objects which can be recognised by reference to a plate. An attempt is made here to describe and so far as possible illustrate such deposits as are likely to be met with in the urine.

If a considerable deposit is present in the specimen glass do not centrifuge. Draw up a portion of the deposit in a pipette. If the deposit is scanty or absent wash out thoroughly two centrifuge tubes. Shake up the bottom portion of the specimen after carefully pouring off the supernatant fluid. Fill the centrifuge tubes with urine and centrifuge for a few minutes at a moderate speed. Do not stop the centrifuge after turning off the power but allow it to run down. Remove the tubes and make use of the one that contains the more deposit. Turn the tube upside down the bulk of the deposit will

remain in the last drop which will not fall out. Prepare a clean slide and cover glass. Shake out a drop of the urine containing the deposit on the centre of the slide. *Let down a cover glass on the slide.* If the right amount of deposit is taken there will be no air bubbles and the urine will not spread over the slide beyond the cover glass. Examine with the microscope vertical and the diaphragm partly closed. Use both a $\frac{2}{3}$ inch and a $\frac{1}{4}$ inch objective.

Blood. Red blood corpuscles are recognised under the higher objective by their shape, size and colour. The size and shape are not infrequently altered by crenation. The colour is very distinctive. With practice a single red cell can be recognised with certainty. The objects most frequently confounded with red blood corpuscles are oil globules and ammonium urate spheres. Oil globules are often seen in the deposits of catheter specimens and are derived from the lubricant used for the catheter. They are more circular than red cells and of very varying sizes from circles smaller than red cells to obvious large globules. They are yellowish in colour but of a different tint from the corpuscles. Uric acid crystals mislead only by their colour. They can be recognised by their regular outlines and crystalline shape.

Should there be any doubt as to the nature of the corpuscles a film preparation should be made and stained with Leishman's stain.

Blood may also appear in the urine as a contamination from some other part of the body and in cases of doubt particularly in specimens obtained from women a catheter specimen should be examined.

The presence of blood in the urine will probably have been confirmed by the guaiac test or by the spectroscope. The amount of blood which can be detected by the microscope may be so small as to escape observation by the other tests.

In all cases of hæmaturia an estimate should be made of the relative proportions of red cells and hæmoglobin present. There is no difficulty in this. If sufficient blood is present to colour the urine obviously a very large number of red cells will be present in the deposit. In cases of hæmoglobinuria red cells will be almost entirely absent and broken up into granular masses often in the shape of casts.

Pus (Plate X.) Pus corpuscles are phagocytic cells and as such consist of polymorphonuclear neutrophils with occasional

large hyaline cells and epithelial cells. The corpuscles may be more or less degenerated but the majority of cells in most samples of pus are perfectly well formed and normal in appearance. There is no actual dividing line between a polymorphonuclear cell and a pus corpuscle. If polymorphonuclear cells are sufficiently numerous to produce a naked-eye deposit pus is present. If few cells are found the process is the same and the difference is one of degree only.

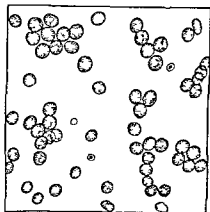
Urines containing pus cells in large numbers give a greenish colour with the guaiac test but the microscope is the only accurate method of detecting pus. The performance of the potash test for pus is a waste of time and is not described here.

Pus corpuscles are readily recognised in unstained specimens by their size, their round shape, the commonly bilobed or polymorphonuclear form of their nuclei and by their refractile granular appearance.

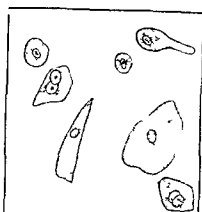
When a considerable degree of pyuria is present the pus may be partially obscured by the presence of mucus in addition. The pus in such cases is very viscid and difficult to control when preparing a microscopic specimen. When viewed under the microscope the cells are partially hidden under the mucus and can only be seen by careful focussing. Such specimens floated in water may resemble membranes in appearance and may have the shape of the bladder or urethra. Prostatic casts are of similar composition.

In cases of doubt a thin film should be spread on a slide with a platinum wire, dried, stained for 3 minutes with carbol thionin, washed in water, blotted dry and examined with an immersion lens or even with a $\frac{1}{6}$ inch objective after mounting in Canada balsam. The polymorphonuclear cells are often somewhat shrunken in such specimens but are perfectly recognisable and in addition any bacteria which may be present can be noted.

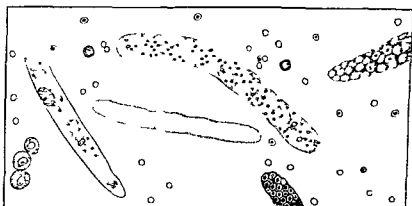
It is occasionally necessary to determine if a urine which contains blood has pus present in addition since polymorphonuclear cells are necessarily found when the bleeding has been free. The question is fairly easily answered by the microscopic examination of the deposit. In the case of blood the average field of the $\frac{1}{6}$ inch objective shows large numbers of red cells and 1 or at most 2 or 3 leucocytes. If pus is present in addition the field will show a dozen or more leucocytes among the red cells and fields will be found in which the



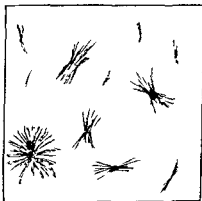
Pus Corpuscles
(Urinary Deposit)



Epithelial Cells
(Urinary Deposit)



Casts, etc
(From the Deposit of a Case of Acute Nephritis)



Tyrosine Crystals
(From Urine, after Separation)



Spermatozoa.
(From a Spermatocele)
(Stained Specimen)

leucocytes are gathered in small clusters. Conversely in cases of considerable pyuria occasional red cells will usually be detected among the leucocytes.

The presence of polymorphonuclear leucocytes in a urinary deposit is pathological provided that they came from some part of the urinary tract. It is essential for the detection of small traces of pus in the urine of women that a catheter specimen should be examined. In men the pus may come from the urethra in cases of gonorrhœa and it is not infrequent to find a few leucocytes probably derived from the prostatic urethra in the urine of patients who have had gonorrhœa many years previously. The pus in such cases may or may not be due to the actual presence of gonococci. The pus cells are frequently united by mucus into long thread like processes visible to the naked eye and known as prostatic threads. These threads are most often present in the first specimen of urine passed in the morning and may be seen best after massage of the prostate.

Epithelium (Plate X) Epithelial cells are commonly present in the urine and in deposits taken from the urine of female patients are as a rule very abundant. Epithelial cells in the urine have no pathological significance but must be recognised since they are apt to be mistaken for pus corpuscles or even portions of new growth.

Epithelial cells may be recognised by their size being commonly much larger than the polymorphonuclear cells by their shape which is rarely round by the absence of granularity and by their single central nucleus. They cannot be distinguished from the single cells of a neoplasm.

Epithelial cells differ considerably in shape and size and the student is commonly asked to recognise from the nature of the cell that portion of the urinary tract from which it is derived. We are by no means convinced that this is possible. The commonest form of epithelial cell is a very large angular cell of the squamous type. It is the predominant cell in the urine of women and may be entirely derived from the vagina. Smaller pear shaped and tailed cells are often predominant in ureteric specimens and less commonly small round cells which closely resemble polymorphonuclears but differ from them in having a round nucleus. Such cells are presumably derived from the ureter or pelvis of the kidney. The ureteric lining is very friable and readily damaged and specimens obtained by ureteric catheterisation are frequently grossly contaminated by

blood It is only in very skilled hands that epithelial cells only are rubbed off in normal cases, and these are commonly present in abundance and have to be carefully distinguished from pus corpuscles

New growths In descriptions of the methods of diagnosing new growths of the bladder or kidney the detection of particles of growth in the urine or in the eye of the catheter holds a time honoured place It is perfectly reasonable to look for such particles but it is extremely rarely that one is able to identify them The recognition of a single cell or even a small cluster of cells from a neoplasm is quite impossible The more solid fragments in such urines nearly always turn out to be blood clots Fragments of growth may, if present be recognised after teasing out if necessary, and flattening in a drop of salt solution between a slide and cover slip A drop of dilute methylene blue may be allowed to run under the cover slip if desired for staining purposes Definite villus like papillary processes lined with epithelium can only come from a neoplasm Such processes have to be distinguished from particles of pus and epithelial cells bound together by "mucus" Pus of this description presents an undulating outline but no regular processes Actual naked-eye fragments of a papilloma or carcinoma are in cases of doubt preferably fixed and sectioned The distinction between a simple and a malignant growth in such chance particles may be impossible

Fæcal elements Fæcal elements may be present in the urine particularly of female patients as a contamination The student on examining the urinary deposit of a catheter specimen and being confronted with striated muscle fibres, may be puzzled to account for them Striated muscle fibres are yellow in colour, and show more or less clearly the transverse striping They are quite unmistakable and in a catheter specimen are definite evidence of abnormal communication between bladder and intestine The most common cause of such communication is a recto vesical fistula which may first attract attention by the production of a cystitis If muscle fibres are absent, vegetable fibres may be recognised by their shape and spiral appearance and in the absence of these elements fæcal contamination can be inferred only from the smell and the general heterogeneous appearance of the deposit

Casts (Plate X) Casts are among the most important of the formed elements in the urine If casts are present in

any numbers and of certain varieties we can be satisfied that some form of nephritis is present and can sometimes determine which form. Tube casts are products of the cells lining the renal tubules, and their permanent form and outline are probably due to the fact that they have been formed in the tubules and have remained some time *in situ*. If the tubular epithelium is normal and the renal tubules are properly patent, no deposition of casts can take place. The presence of tubular casts in the urine is consequently evidence of renal disease.

Castes vary greatly in size and to a less extent in shape. A cast of average size is just recognisable under a $\frac{2}{3}$ inch objective, and when only a few casts are present they are best searched for with this power. A $\frac{1}{2}$ inch objective is necessary for their verification, and should always be used in addition. Casts are recognised by the nature of their contents which will be subsequently described, but particularly by their shape and their definite outline. In shape casts are long and more or less narrow cylinders with rounded ends. Occasionally one end is rounded the other ragged as if fractured. A cast has a clear cut and sharply defined outline. The limiting edge of the cast distinguishes it from the only objects that could reasonably be mistaken for it. Amorphous urates, and less commonly amorphous phosphates, may be found in little masses, which exactly resemble casts in shape and for the reason in some cases that they are probably formed in the renal tubules. Such uratic casts are distinguished in a careful examination by the comparative irregularity of their edges and the absence of a definite outline. Casts on the whole are remarkably like the representations of them in many text books, and if the beginner sees a well formed cast he almost always recognises it. The chief objects that may be mistaken for casts are the aggregations of urates already mentioned, hairs, and wool, cotton fibres, rolled up epithelial cells and especially cylindroids. Hair and wool can usually be distinguished by their transverse imbrications, and cotton by its longitudinal striations. If the student is in doubt as to whether the object he sees is a cast or not, it may be anything from an epithelial cell to a scratch on the glass, but it is very rarely a cast.

Castes are of several varieties, each of which has a somewhat different significance. The casts are divided according to their structure into cellular, granular, and amorphous varieties.

Cellular casts are further subdivided according to the nature of the cells present into epithelial erythrocytic, and leucocytic casts. Epithelial casts contain the mononuclear cells of the shed renal epithelium. The epithelial cells may be more or less degenerated, and their nuclei may be absent. Some cells may appear vacuolated, others granular. Some may contain globules of fat. The cast may be entirely filled with cells or may be partly hyaline. Erythrocytic casts are readily identified and may consist of casts packed with obvious and undegenerated red cells. Some casts may contain both red cells and epithelial cells and the red cells may be more or less broken up. These casts are similarly found in acute nephritis and particularly in the acute stages. Free red cells are always present in the urine in addition. Leucocytic casts, that is casts which contain polymorphonuclear neutrophils, are not commonly met with. They may be found in septic conditions of the kidney, such as "surgical" or "consecutive" nephritis. A few polymorphonuclear cells are nearly always present in addition to the casts in a case of acute nephritis, and a few degenerated epithelial cells are also seen lying free.

Granular casts are casts which contain no formed elements other than fine or more rarely coarse, granules. Granular casts are perhaps the most common variety seen and usually predominate in chronic parenchymatous nephritis as well as in acute nephritis after the first few days of the disease. The majority of granular casts are probably derived in the same way as the epithelial casts and differ only in representing a later stage in the cells which have now undergone complete granular degeneration. In the terminal stages of nephritis a large opaque dark cast is often found. Granular casts may less frequently arise from a granular change in the amorphous forms.

Amorphous casts The commonest variety of amorphous cast is the *hyaline cast*. This varies considerably in size but is usually smaller than the granular cast. The content of the cast is as a rule quite structureless and almost entirely transparent. These casts may escape observation unless the light reflected through the preparation is reduced to a minimum. Hyaline casts may be found in every form of nephritis, and may be the only variety present in cases of chronic interstitial nephritis and kidney damage in hyperpiesia. Very occasionally a cast of this kind may be found in the urine of an apparently healthy person.

Colloid casts are larger and more opaque than hyaline casts. They are found particularly in nephrosis.

Amyloid casts, consisting of amyloid, are said to occur in the urine in amyloid degeneration of the kidneys. They are very rarely found.

Cylindroids are long pale ribbon like bodies consisting of mucus showing fine longitudinal striation, usually tapering, and often frayed out at the ends. They are found particularly in inflammations of the urinary tract. They may be covered with granules (urates, bacteria, crystals, cells, etc.) in which case they may resemble granular casts.

Spermatozoa (Plate X) are occasionally found in the urine, and should be readily recognised by their shape. The long, thin and tapering tail with the prominent oval head, are quite unmistakable. Spermatozoa in the urine may show active movement even in a specimen which has been standing for some time. Spermatozoa are readily stained by the ordinary dyes such as carbol thionin.

Bacteria may be seen in urinary deposits with the $\frac{1}{2}$ inch objective. No significance should be attached to their presence except in freshly passed urine or urine collected with aseptic precautions.

Animal parasites are not commonly met with in the urine of patients in this country. Occasionally the common thread worm (*Oxyuris vermicularis*) or its ova may be found in the urine of little girls. The only parasitic infection of the urinary tract at all commonly met with is that of *Schistosoma hæmatobium*. In cases of hæmaturia coming from tropical or sub tropical climates such as Egypt or parts of South Africa where this parasite is numerous its presence should always be suspected. Infection of the bladder by schistosoma is recognised with certainty by the detection of the characteristic ova in the urine. When hæmaturia is present the ova are generally numerous and they may persist for many months after residence in an infected district. A specimen of the urinary deposit should be put up in the ordinary way, and the ova can readily be seen with the low power of the microscope and the details of the contained embryo can often be made out on using the $\frac{1}{2}$ inch objective. If the ova are very scanty the patient should be told to empty his bladder, and then to pass the last few drops of urine evacuated by active straining into another receptacle. The ova are large

oval bodies with a definite capsule terminating in a single sharp, short spine which is quite characteristic. Within the ovum can often be seen a coiled, ciliated embryo which may show active movement. If fresh water is added to the urine the embryo may burst its way out of the ovum and swim about freely.

UNORGANISED URINARY DEPOSITS

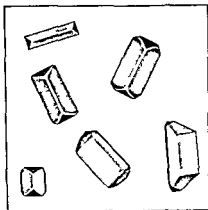
While the greater number of the organised urinary deposits are only present in the urine as the result of disease the majority of the unorganised deposits are not necessarily of any pathological import. The detection of a crystalline or non-crystalline substance in the urine is no evidence that it is being excreted in excess. The only conclusion that can be drawn is, that the sample of urine on cooling and standing is unable to contain the substance in solution. The majority of normal urines and almost all concentrated urines form some deposit on standing. Those urines from which no deposit can be obtained, even after standing and centrifuging, are nearly always derived from cases of polyuria. The detection of uric acid or calcium oxalate crystals in the urine of a patient with renal symptoms is very little evidence that a calculus of similar composition will be found in the kidney.

The more important of the unorganised deposits are most conveniently classed into those found in acid and those found in alkaline urine, this assists memory, as there are five in each group.

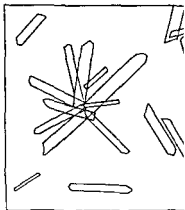
In Acid Urine

Urates (Plate XI) which consist mainly of acid sodium urate are amorphous brown or pink soluble on warming. They are very common and occur in large amounts in the urine of patients with heart failure and fevers, also in the urine of healthy persons who have sweated profusely, especially if the urine gets very cold on standing. A concentrated highly acid urine in a specimen glass may throw down several inches of urates.

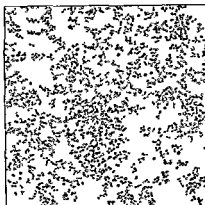
Uric acid (Plate XII) appears as crystals which are usually coloured yellow or brown are soluble in sodium hydroxide, and reprecipitated by hydrochloric acid. The forms assumed by the crystals are very varied, the whetstone shape as shown



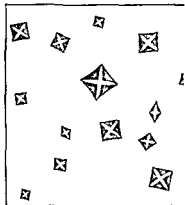
Triple Phosphate Crystals



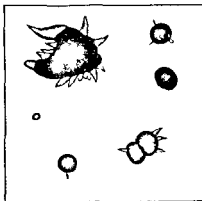
Calcium Hydrogen Phosphate Crystals



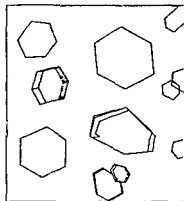
Amorphous Urates



Calcium Oxalate Crystals



Ammonium Urate Crystals



Cystine Crystals

in the figure is one of the most common, rhombic prisms, wedges, rosettes, dumb bells, butcher's trays are also common. Not uncommon is the deposition of uric acid in granules visible to the naked eye and the colour of cayenne pepper. These granules, if passed in considerable amount, are referred to by the patient as 'gravel'. They consist of clusters of crystals resembling rock candy. Colourless crystals of uric acid are described, but one of the forms figured in books is really rye starch. Uric acid crystals dissolve on warming with sodium hydroxide.

Deposits of uric acid occur in much the same conditions as do deposits of amorphous urates.

Calcium oxalate (Plate XI) occurs in small clear colourless octohedral crystals with an envelope appearance, also in dumb bells. Usually the crystals are only just visible under the $\frac{2}{3}$ inch objective, and the $\frac{1}{8}$ inch is required for identification, they are insoluble in acetic, but readily soluble in hydrochloric acid. Although more commonly found in acid urine, they may occur in alkaline. These crystals are common in perfectly normal urine, larger numbers occur after taking certain fruits and vegetables particularly rhubarb.

Stellar phosphates (Plate XI) (calcium hydrogen phosphate) are found in nearly neutral urine, they are readily soluble in acids. The crystals are clear and colourless, most commonly they form long, narrow flat prisms which are frequently collected together in bunches or rosettes. Less often the crystals may be fine and feathery and collected into tufts.

Cystine (Plate XI) is found as colourless hexagonal crystals, which have clearly cut straight sides, the sides may be unequal but the angles are those of a regular hexagon. The crystals are insoluble in acetic acid and ether soluble in hydrochloric acid and in ammonia and other alkalies, the crystals do not occur in alkaline urine. They are very rarely met with, but are included in this list, owing to their clinical importance, as their occurrence may be the sole evidence of a rare congenital abnormality, cystinuria. This condition would be of theoretical interest only were it not that the cystine may form calculi in the kidney and bladder.

To examine an alkaline urine for cystine, make acid with acetic acid, allow to stand overnight, and examine the deposit.

In Alkaline Urine

Ammonium urate (Plate XI) forms a yellow or brownish deposit, soluble on warming. Under the microscope the deposit may be amorphous or in granules visible under the $\frac{3}{8}$ inch objective, of irregular shape, with one or more spinous processes (thorn apple). This deposit is common in concentrated alkaline urines.

Amorphous phosphates (calcium and magnesium phosphate) form a white amorphous deposit, soluble in acids. This deposit cannot be distinguished from pus, with which it is often mixed, by naked-eye examination, under the microscope it is seen to consist of white amorphous granules.

Triple phosphates (Plate XI) (ammonium magnesium phosphate) form clear, colourless crystals, visible with the $\frac{2}{3}$ inch objective, and sometimes with the naked eye, soluble in acids. The crystals assume various forms, it is usually easy to find the typical "knife rest" forms shown in the figure. All alkaline urines deposit amorphous or triple phosphates, unless they are deficient in calcium, magnesium, or phosphates, the presence of these deposits has no significance other than that of an alkaline urine.

Stellar phosphates also occur in faintly alkaline urine.

Calcium carbonate forms a white deposit which may be amorphous or crystalline, the crystals having the shape of dumb bells. It dissolves in acid with an effervescence, which distinguishes it from amorphous phosphate, with which it is often mixed. It occurs chiefly in the urine of herbivora, but also in stale and very alkaline human urine.

Besides these more common deposits the following may be mentioned —

Tyrosine (Plate X) may occur together with *leucine* as a deposit in the urine in atrophy of the liver, but these two deposits are not common even in this condition. Tyrosine is less soluble than leucine and separates out more readily, it forms brush like tufts of fine needles, which are colourless or greenish yellow and are soluble in ammonia and hydrochloric acid insoluble in acetic acid.

Leucine crystals take the form of yellow spheroids, which show both radial and concentric striation. Leucine is readily soluble in acids and alkalis.

Mucin is not the common deposit in urines that the examination of case sheets would lead one to believe. One of the

deposits most commonly mis-called mucin is a haze of bacteria. The occurrence of mucin is described in the next chapter.

Foreign Bodies in Urinary Deposits

A complete list of the adventitious material which may be found in the urine would rival the well known list of foreign bodies met with in the vagina though there is no record of a bust of Napoleon in a specimen glass. Patients may however deposit almost any available object in the urine. The following include some of the more common adventitious objects which may be puzzling to the student.

Marks on the cover-glass have been frequently and earnestly scrutinised with a view to interpretation and the mistake is easily made. If the cover glass is not scrupulously clean and it is difficult to make it so or if it is scratched the dust and scratches on its surface are the objects first seen on focussing the objective down. The scratches may assume fantastic shapes and may even remotely resemble casts while the surrounding dust is mistaken for the urinary debris. The mistake is at once rectified by continuing to focus down until the actual urinary deposit comes into view. Scratches on the slide itself may of course be similarly mistaken for urinary contents and have to be differentiated by their shape and appearance. They present no real difficulty.

Air bubbles should not be present in a carefully put up sample of deposit still they sometimes occur. They are circular in form unless compressed. They are recognised by their broad dark and often double outlines and their clear shining centres.

Oil globules may occur in disease but are more frequently present as a foreign body in catheter specimens. They are recognised by their circular form their variability in size their sharp margins and refractile centres. They turn black on adding osmic acid.

Starch granules are fairly frequently found in the urine particularly of children their source being the dusting powder used. They turn blue on the addition of iodine.

Sulphur granules are occasionally found in the deposit of urine which has been subjected to the sulphur test for bile salts. The granules appear as dark irregular clumps of crystalline bodies.

Hairs are commonly present in the specimen glass and are

usually pretty obvious to the naked eye. They are as a rule pigmented and their structure becomes more obvious on the addition of caustic soda.

Fibres of cotton or linen are very commonly met with, and are usually derived from the cloth used in cleaning the specimen glass. They are necessarily of variable size and shape but are all more or less cylindrical and twisted, and usually have frayed ends. The only pathological objects for which they can be mistaken are casts, and the resemblance is not particularly close.

CHAPTER XVI

FURTHER EXAMINATION—QUANTITATIVE METHODS

Abnormal Pigments

Urobilin and its colourless chromogen *urobilinogen* are derived from the reduced bilirubin (stercobilin) in the intestine, some of which is absorbed. Normally most of this urobilin is re-excreted by the liver and only a small amount reaches the kidneys and appears in the urine mainly as urobilinogen. Abnormally large amounts of the pigment and its chromogen may be passed in the urine. This increase may follow an excessive destruction of red cells, as in pernicious anæmia, leading to an excessive excretion of bilirubin into the intestine with increased absorption, or there may be failure of excretion, as in diseases of the liver. The abnormal excretion in fevers is probably due to liver damage.

Increased amounts of urobilin give to the urine a rather more pink shade of orange than does mere concentration.

If urobilin and urobilinogen are present in considerable excess the absorption band at the junction of the green and blue can be seen with a direct vision spectroscope, after the addition of 3 drops of tincture of iodine to 10 c.c. of urine to convert the urobilinogen present into urobilin (Fig. 7). Normal urine shows no bands, but a general darkening of the violet end of the spectrum.

A more delicate test for urobilin and urobilinogen is performed as follows. Add iodine to the urine as before, and if the urine is alkaline make slightly acid with acetic acid. In another test tube shake 1 gram of zinc acetate with 10 c.c. of absolute alcohol. Pour the urine into this second test tube and pour the mixture from one tube to another until most of the zinc acetate has dissolved, filter into another test tube and examine against a dark background with the light falling from behind. A green fluorescence occurs if urobilin or urobilinogen is present and the fluid is pinkish by transmitted light, with normal urines the fluorescence is very faint.

Uroerythrin a pink pigment is very commonly absorbed on deposits of urates in urine. It has been said to indicate derangement of the liver but is very common in the urines of healthy persons. On adding sodium hydrate to the deposit the pigment turns green. Very occasionally urines are coloured throughout with uroerythrin which may be recognised by the fact that it turns green on adding alkali.

Untreated urines pigmented with indigo blue have been described but are very rare.

✓ *Indican* (indoxyl hydrogen sulphate) occurs in most urines. It is colourless but with strong acids containing weak oxidising agents forms indigo blue and indigo red. Indican in urine can be demonstrated as follows —

Measure 5 c.c. of urine into a test tube and add 5 c.c. of strong hydrochloric acid to which just sufficient ferric chloride solution has been added to give it a faint yellow tinge. Let stand half an hour. add 5 c.c. of chloroform and extract by inverting cautiously ten times. If indican is present in the urine the chloroform is coloured blue.

As might be expected concentrated urines usually give strong indican reactions. allowance can be made for this by always using for the test $\frac{1}{300}$ th of the total 24 hours urine. The test has been regarded as a measure of intestinal putrefaction but no convincing proof of this has been brought forward.

Urorosein is a red pigment formed from a colourless chromogen when urine is heated with strong hydrochloric acid. It is insoluble in chloroform soluble in amyl alcohol and gives an absorption band in the green. The colourless chromogen is present in most urines. the occurrence of the pigment readily formed in urine has been described but is at any rate extremely rare.

Homogentisic acid is found in urine in the rare congenital condition alkaptonuria. The urine has a normal colour when passed but turns brown to black on standing particularly if the urine becomes alkaline. Homogentisic acid reduces Fehling's solution producing a yellow to brown colour throughout the fluid with little deposit. it gives a striking transient blue colour on the addition of ferric chloride solution.

Carboluria may occur in any variety of carbolic acid poisoning. With susceptible patients the absorption of a very small quantity of carbolic acid such as takes place after

the application of a 'carbolic cap' to the head may lead to the production of carboloria. The patient may show little or no symptoms. The urine becomes considerably darker on standing or after the addition of nitric acid and in well marked cases is of a distinctive greenish blue colour. This colour and the evidence of exposure to carbolic acid helps to distinguish the urine from that of melaninuria. The pigments produced in the urine by carbolic poisoning are chemically similar to those of alkaptonuria. Fehling's solution may be reduced.

Melaninuria is a rare condition which may be found in patients with melanotic sarcoma and occasionally with non-malignant pigmented papillomata of the skin. The urine as a rule is dark when passed but becomes considerably darker on exposure to the air. The addition of a few drops of nitric acid or bromine water causes immediate blackening of the urine. No reduction of Fehling's solution is produced and no reaction with the guaiac test. No absorption bands are seen with the spectroscope. A dark urine which fulfils these conditions in all probability contains melanin but there is no simple and satisfactory direct test for this substance.

Hæmatoporphyrin or an allied pigment is present in normal urine but only in very small amount. A slight increase may occur in various diseases.

Great increase causing a change in the colour of the urine occurs as an acute condition in poisoning with sulphonal and allied drugs. From this cause large numbers of cases have occurred in lunatic asylums. Unless the condition is recognised at once and the sulphonal stopped death is likely to result. Acute cases also occur where no drug can be incriminated. *Hæmatoporphyrinuria* also occurs very rarely as a chronic condition apparently congenital. These cases are particularly liable to *hydroa aestivale*.

The pigment in cases in which large amounts are present in the urine differs in composition from hæmatoporphyrin as usually prepared from hæmoglobin. When the pigment is in great excess in urine it may be recognised by direct examination with a spectroscope. The spectrum is the same as that of alkaline hæmatoporphyrin (Fig 7) i.e. four bands one between C and D one at D one just on the red side of E and a broad band between the green and the blue. If the spectrum

is not clearly obtained the pigment should be extracted, before examination, as follows —

To 500 c c of urine add 5 c c of 40 per cent caustic soda. Allow the deposit of phosphates on which the pigment is absorbed to settle. Decant the supernatant liquid, wash the precipitate with water on a filter paper, extract with alcohol acidified with hydrochloric acid and examine spectroscopically. The pigment will then give the spectrum of acid hæmatoporphyrin—a narrow band in the orange near the D line, and a broad band in the yellow.

If a satisfactory precipitate is not obtained on adding NaOH, dissolve 0.5 gram of calcium chloride and of acid sodium phosphate in separate test tubes and add to the alkaline urine, stirring meanwhile.

Urine containing this pigment do not reduce Fehling's solution nor give the guaiac reaction.

Methylene-blue taken by the mouth, or given hypodermically, leads to the production of a remarkable bright green (not blue) urine. The coloration of the urine may persist for several days after taking the drug, and is quite harmless. The mental shock to a neurotic patient on passing bright green water may, however, be considerable.

Eosin has been extensively used as a colouring matter for cheap sweets, and a curiously dichroic urine may result. The tint of the urine is exactly that of a dilute solution of eosin and is easily recognised.

Among other substances *santonin* may produce a greenish urine, and *rhubarb* or *senna* a reddish brown coloration. *Resorcin* used as an ointment may lead to a striking greenish coloration of the urine.

After taking *pyramidon* a cherry red colour, which becomes deeper on standing may appear in the urine.

Phenolphthalein may be administered as a laxative. It gives a red colour to urine if this is sufficiently alkaline. The colour disappears on acidifying and on shaking with ether, as the ether extracts the phenolphthalein in the colourless form.

Proteins

The proteins in urine usually called "albumin" are identical with the albumin and globulin of normal serum. Other proteins may be found in urine.

Proteoses have been detected in the urine in a variety of conditions. Tests for them are unsatisfactory, and at present no clinical significance can be attached to them.

Bence Jones protein and allied proteins may be found, but are not invariably present, in the urine of patients with multiple myelomata. These proteins occur rarely, if ever, in any other condition. Their common peculiarity lies in coagulating at a lower temperature (about 55°) than the usual coagulable proteins of urine. Typical Bence Jones protein also has the striking property of going into solution again at about 100° , if the concentration of salts in the urine is suitable, as is usually the case. Allied proteins instead of re-dissolving, form a rubber-like mass when the urine is brought to the boil, very different from the coagulum formed by albumin and globulin.

To test urine for these proteins take 10 c.c. of urine, made slightly acid with acetic acid in a test tube, add 1 c.c. of 7 per cent calcium chloride, place a thermometer in the test tube and stand the tube in a beaker of water. Heat the water slowly and note the temperature at which the protein begins to coagulate. If this is under 60° , the protein is probably one of the Bence Jones group. This will be confirmed by the solution of the protein or formation of a rubbery mass when the water is brought to boiling.

Hydrochloric acid added to the urine produces a precipitate, even when only small quantities of these proteins are present, the precipitate dissolves completely on warming.

Bence Jones protein is frequently spoken of as an albumose, but it more closely resembles a globulin.

Mucin appears in the urine with inflammation of the urinary tract and more particularly from contamination with mucin from the vagina or prostate. It forms a cloud at the bottom of the glass, is precipitated by acetic acid as a stringy mass, insoluble in excess and is not coagulated by boiling.

Nucleoproteins appear in small quantities in the urine mainly from the breakdown of leucocytes, they are coagulated by boiling, but may be distinguished from albumin and globulin by the fact that they give with acetic acid in the cold a precipitate soluble in excess.

Cystine. If a patient is suspected of cystinuria, but no crystals are found in the urine the following test may be tried. To 3 c.c. of urine add 2 c.c. of 4 per cent sodium cyanide solution. Stand 5 minutes and add a few drops of 5 per cent

sodium nitroprusside solution. A stable magenta colour appears to indicate the presence of an abnormal sulphur compound not necessarily free cystin. Creatin and acetone give only very faint colours.

Further Investigation of Reducing Substances

Glucose appears in the urine when the blood sugar is raised or when the renal threshold is abnormally low (renal glycosuria), the distinction between these conditions is given later (p. 364). Temporary glycosuria of the first type may be produced by morphia and anæsthetics.

The amount of glucose found in the urine in true diabetes mellitus varies greatly with treatment, in untreated cases 200 or 300 grams may be passed in 24 hours, and the concentration may be as high as 12 per cent. In renal glycosuria the amount passed is never large, so that the concentration found in urine is not more than about 1 per cent.

The following are the chief reducing substances other than glucose that may be found in urine.

Lævulose occurs in the urine after large doses of levulose, and in small quantities together with glucose, in some cases of diabetes mellitus and renal glycosuria. Lævulose is fermented by yeast and its osazone is identical with that of glucose, the presence of lævulose in urine can be detected by *Selvanoff's reaction*.

Selvanoff's reagent. Dissolve 0.05 gram of resorcinol in 100 c.c. of concentrated hydrochloric acid and dilute with 100 c.c. of distilled water. To 5 c.c. of the reagent add a few drops of the urine and heat. If levulose is present a red colour and red precipitate appear. The precipitate dissolves in alcohol giving a red solution.

A red colour is also given by glucose after long boiling but a precipitate is not formed.

Lactose occurs in the urine of pregnant and of lactating women, especially if they are not suckling their babies in amounts up to about 1 per cent. There is no convenient test for lactose, its osazone, when obtained in a crystalline form, appears as needles radiating from the centre of a sphere, looking rather like the head of a chrysanthemum. Lactose is not fermented by yeast.

Pentoses. The appearance of these in the urine is the

essential feature of a very rare congenital abnormality called Pentosuria. Large quantities are not excreted. There is some doubt about the actual amounts, owing to controversy concerning the method of estimation, but the result of Fehling's test resembles that obtained with about 0.5 per cent. of glucose.

Pentoses are not fermented by yeast, their osazones, when obtained, form very fine needles arranged in a felt work, the presence of pentoses may be detected by *Bial's orcinol test*.

Bial's reagent. Dissolve 1 gram of orcinol* in 500 c.c. of concentrated hydrochloric acid (specific gravity, 1.151), and add 25 drops of 10 per cent. ferric chloride solution. This reagent should be kept in an amber bottle.

Boil 5 c.c. of this reagent in a test tube, remove from the flame and run 0.2 c.c. of the urine from a pipette on to the surface of the reagent. If pentoses are present a green ring appears which spreads through the liquid on careful shaking, with a spectroscope an absorption band can be seen in the yellow. The colour can be extracted with amyl alcohol.

Glycuronic acid is normally present in urine in amounts insufficient to reduce Fehling's solution, increased quantities are excreted after the ingestion of phenol derivatives (e.g., salicylic acid) and camphor. The amounts are never large, so that the reduction of Fehling's solution is never more than that produced by 1 per cent. glucose. Glycuronic acid is not fermented by yeast, its osazone, if obtained, resembles those of pentoses.

Homogentisic acid (further discussed under abnormal pigments in urine) occurs in urine in the rare congenital abnormality alkaptonuria. The reduction of Fehling's solution is peculiar, the amount of precipitate formed is small, and the whole liquid turns a yellowish brown. Homogentisic acid is not fermented by yeast and does not give an osazone.

Uric acid in concentrated urines may give a greenish precipitate with Fehling's solution. It does not reduce Benedict's solution, does not give an osazone, and is not fermented by yeast.

Methods of Identification

When it is necessary to consider the question left over from the last chapter "What is the reducing substance in urine?" no help is given by the appearance produced by Fehling's or

* The word "orcein" is used for orcinol and for another substance.

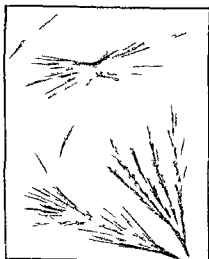
Benedict's tests, except in the case of homogentisic acid the appearance produced depends solely on the amount of reducing substance present, and even the appearance produced by homogentisic acid may be simulated by glucose under special circumstances. Nor is the multiplication of tests similar in principle to Fehling's of any assistance. Two methods commonly used are the appearance of the phenyl-osazone of the reducing substance and the behaviour with yeast.

To prepare the *osazone*, fill a test tube two thirds full of urine, add as much phenylhydrazine hydrochloride as will be on a sixpence twice as much sodium acetate, and make strongly acid with acetic acid. Warm in hot water and shake until the solids have dissolved, then hang in a boiling water bath for half an hour. Allow to cool, place a drop of deposit on a slide, cover with a cover-slip and examine under the low power of the microscope. If glucose is present in large amounts (2 per cent or more) the osazone usually precipitates soon after the tube is removed from the water bath, and will be seen to have the typical appearance—sheaves of needles (Plate XII). When the amount present is less the precipitation is slower and the crystals less typical, forming rosettes or spheres with spikes sticking out and indistinguishable from lactosazone (Plate XII). When the amount of glucose present is small no osazone may be obtained.

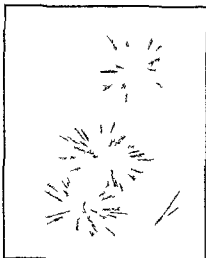
The osazones of the other sugars and glycuronic acid are less easily obtained, and uric acid does not form an osazone. The test is chiefly of value when a typical glucosazone is obtained, in this case the reducing substance may be pronounced with confidence to be glucose.

The *yeast fermentation* test is performed as follows. Heat about 40 c.c. of urine in a boiling water bath to kill bacteria. Cool, make slightly acid if necessary, with acetic acid. Reserve about 10 c.c. and rub up the rest with a piece, about as big as a hazel nut, of German yeast, such as can be obtained from a baker. Place the mixture in a Doremus ureometer tube so as to fill completely the long limb of the tube and about half the bulb, and to leave no air bubbles at the top of the tube. The tube is placed in a warm place, preferably in an incubator at 37°, and left. It is as well to put up two control tubes, one containing yeast and water only, since a small quantity of gas may be given off from the yeast, the other containing yeast and a glucose solution, to make sure that the yeast is active.

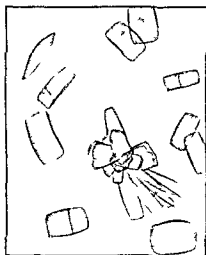
PLATE XII



Chloraz Crystals



Lactose Crystals



Urethral Crystals

After 24 hours examine the tube for bubbles of gas, which will show that fermentation has taken place. Filter the contents of the tube and test the filtrate and, for comparison, the reserved urine, with Fehling's solution. If gas has been formed and the reducing substance removed, this reducing substance is glucose or laevulose. If the amount of reducing substance may be insufficient to form a gas bubble the disappearance of the reducing substance after incubation may be considered sufficient evidence that it is glucose or laevulose. The other reducing substances enumerated are not fermented. The Doremus ureometer tube may be replaced by a test tube, completely filled and carefully inverted with its mouth immersed in a beaker of water.

It is often difficult to be certain whether a reducing substance present in small amount in urine is glucose or not, the glucose tolerance test is a great help in deciding this question.

If glucose is found in the urine it is still necessary to consider whether its presence is due to impairment of the power to metabolise carbohydrates, or to an abnormally low renal threshold for glucose (renal glycosuria).

Renal glycosuria may be distinguished from fully developed diabetes mellitus by clinical methods alone, but to distinguish it from moderate degrees of impairment of the power to metabolise carbohydrates can be done with certainty only with the aid of blood sugar estimations. In all doubtful cases of glycosuria it is advisable to test the power of metabolising glucose by a glucose tolerance test (p. 364).

Quantitative Methods

The composition of the urine to a great extent reflects the composition of the diet, so that quantitative estimations of many of the substances in the urine are in many cases of no value, even for research work, unless the diet is controlled, quantitatively and qualitatively, more carefully than is possible in English hospitals at any rate. The number of quantitative estimations that are of value for clinical purposes are limited, for most purposes quantitative estimations in the blood are much more useful.

When quantitative estimations are made the whole 24 hours' urine must be measured in order that the total amount

of the substances estimated passed in 24 hours can be calculated

Proteins in urine may be estimated as follows —

Principle The urine is mixed with a carbon suspension, and carbon and protein are simultaneously precipitated with trichloroacetic acid. The greyness of the precipitate depends on the amount of protein present. The tone is compared with standards.

Required (1) A series of similar tubes, 3 by $\frac{3}{8}$ inch

(2) Carbon suspension. The most convenient form is a 1 in 25 dilution of "Higgins' Eternal Ink" * in distilled water.

(3) Trichloroacetic acid. Dissolve 100 grams in the minimum amount of water and make up to 100 c.c.

(4) Standards. Solutions are prepared from normal human serum, by dilution, on the assumption that it contains 7 per cent of protein. The solutions are made to contain 0.10, 0.20, 0.30, 0.50, 0.70, 1.0, 1.5 and 2.0 per cent of protein.

One c.c. of each is measured into one of a series of tubes, 0.1 c.c. of (2) and 0.1 c.c. of (3) are added to each, and after vigorous shaking each is corked and labelled. These standards keep 6 months.

Procedure Measure 1 c.c. of urine into a tube. Add 0.1 c.c. of (2) and shake. Add 0.1 c.c. of (3) and shake vigorously. Compare the depth of grey with that of the standards, and read off the per cent of protein.

Estimation of glucose in urine Since the object in the modern treatment of diabetes mellitus is to keep the urine free from glucose or containing only traces thereof, the estimation of glucose in urine is now much less important than it was.

Two methods can be recommended—Gerrard's and Benedict's.

Gerrard's Method

Required. Gerrard's solution. Dilute 100 c.c. of Fehling's solution with 300 c.c. of water, boil and run in 5 per cent solution of potassium cyanide until the blue colour has just gone. Cool, and make up to 500 c.c. with water. This solution will keep several weeks.

Fehling's solution

10 c.c. pipette

25 c.c. burette

* Sold by Chas. M. Higgins & Co. 16 Farringdon Avenue, London, E.C. and 271 Ninth Street, Brooklyn, N.Y., U.S.A.

Round-bottomed hard glass flask

Measuring cylinder

Beaker

Principle Fehling's solution is titrated with the urine, to find the amount that will exactly reduce 10 c c. In order that the end point may not be obscured by cuprous oxide, the double cyanide of potassium and copper (Gerrard's solution), which dissolves cuprous oxide to form a colourless solution, is added.

Procedure If the reduction of Fehling's solution in the qualitative test was strong, dilute the urine one tenth in the measuring cylinder, if the reduction was moderately strong, dilute one fifth and if weak do not dilute at all. Pour the urine, if diluted, into a beaker to mix and fill the burette with it.

Run 10 c c of Fehling's solution into a round bottomed flask add about 30 c c of Gerrard's solution, stand on a round-topped tripod without gauze, and bring to boiling with a naked flame. Keep boiling gently and add urine slowly until the blue colour completely disappears.

Ten c c of Fehling's solution are reduced by 0.05 gram of glucose, so that if x c c of urine diluted one-tenth are required to effect the reduction, $x/10$ c c of urine contain 0.05 gram, and 100 c c contain $0.05 \times \frac{1000}{x}$ grams.

For the estimation to be accurate, between 8 and 13 c c should be required for the titration, if the amount used does not fall within these limits, the estimation should be repeated with the urine diluted less or more than in the first attempt.

If, as tradition still dictates, a porcelain dish is used for the titration, considerable reoxidation of the cuprous oxide formed takes place, so that the figures obtained are too low, the air of a flask is expelled by the steam formed, so that re-oxidation is impossible.

It is convenient to hold the burette in the hand while actually running the urine into the flask.

The amount of glucose passed in 24 hours should always be calculated.

Benedict's Method

Required. Benedict's quantitative solution. With the aid of heat dissolve 200 grams of sodium citrate, 75 grams of

anhydrous sodium carbonate, and 125 grams of potassium thiocyanate in enough distilled water to make about 800 c c of the solution, filter and cool. Dissolve exactly 18 grams of pure crystalline copper sulphate, finely ground in about 100 c c of distilled water and pour it slowly into the first solution with constant stirring. Add 5 c c of 5 per cent solution of potassium ferrocyanide and make the volume up to 1,000 c c with distilled water.

Anhydrous sodium carbonate

150 c c round bottomed hard glass flask

Principle A hot alkaline solution of copper sulphate is titrated with the urine. To prevent the precipitation of cuprous oxide which obscures the end point, potassium thiocyanate is used in making up the copper solution. From the amount of urine required to reduce a known volume of the copper solution the percentage of sugar is calculated.

Procedure Dilute the urine as described under Gerrard's method. Place 3 to 4 grams of anhydrous sodium carbonate (about 1 inch depth in a test tube of $\frac{1}{2}$ inch diameter) in the round bottomed flask. Run in with a pipette 25 c c of Benedict's quantitative solution place on a round retort stand and heat with a naked flame. When most of the carbonate has dissolved run in the urine slowly from a burette, keeping the fluid boiling. When a bulky white precipitate forms, run in the solution more slowly until the blue colour disappears. If less than 5 c c or more than 13 c c of urine are required make up a fresh dilution, such that about 10 c c should be required. The calculation is exactly the same as for Gerrard's method, since 25 c c of Benedict's solution are reduced by 0.05 gram of glucose.

The extra sodium carbonate has to be added, as sufficient will not dissolve in the cold solution.

Urea estimation Interest mainly attaches to the concentration of urea in urine, as discussed under renal efficiency tests, the total amount passed in 24 hours depends on the diet, and usually conveys little information.

Urea may be estimated by the soya bean method or by the hypobromite method. The latter is liable to errors up to 10 per cent, when the urea concentration is some 2 per cent, and to larger errors when the urea concentration is lower, it is much quicker than the soya bean method and may be used when it is necessary to obtain results rapidly.

Soya Bean Method

This is done in the same way as the estimation in the blood (p 76) except that 0.5 c.c. of concentrated or 1 c.c. of dilute urine is used, 5 c.c. of acid phosphate are used instead of 2 c.c. and $\frac{N}{10}$ acid and alkali instead of $\frac{N}{100}$. It is essential to aerate with a rapid air current for at least an hour.

If 0.5 c.c. of urine was used 10 c.c. of acid were taken and x c.c. of alkali were required in the titration the urine contained $2(10 - x) \times 0.3$ grams of urea per 100 c.c. If 1 c.c. of urine was used it contained $(10 - x) \times 0.3$ grams per 100 c.c.

Hypobromite Method

Required Gerrard's ureometer

Alkaline hypobromite solution In a strong glass bottle place 25 c.c. of 40 per cent caustic soda drop a bromine capsule containing 2.5 c.c. of bromine into the bottle fit the stopper tightly and shake till the capsule breaks. Cool before use. This solution can be used twice.

5 c.c. pipette

Principle The urea is decomposed by alkaline hypobromite into nitrogen and carbon dioxide. The carbon dioxide is absorbed by the alkali and the nitrogen given off measured. Theoretically 1 gram of urea should yield 373 c.c. of nitrogen but actually about 354 c.c. are given off. Variation in this latter figure and the evolution of nitrogen from substances other than urea are the chief sources of error in the method. From the amount of nitrogen given off the concentration of urea is calculated.

Procedure Pour water into the wide tube of the ureometer and raise the wide tube until the water in the graduated cylinder is at the zero mark and level with the water in the tube. Place 25 c.c. of the alkaline hypobromite in the bottle of the ureometer. Measure 5 c.c. of urine into the small tube and place this in the bottle carefully so that the urine does not mix with the hypobromite. Cork the bottle tightly open the clip of the T piece to bring the level of the water in the graduated cylinder back to zero. Tilt the bottle so that the urine mixes with the hypobromite. Wait for 15 minutes giving the bottle an occasional shake after the first effervescence has subsided. Lower the wide tube until the water in the tube and

graduated cylinder are again level. Read the level in the graduated cylinder. In most apparatus this gives the percentage of urea directly. If however it gives the number of cubic centimetres of nitrogen given off this multiplied by $\frac{20}{354}$ gives the percentage of urea.

Both these methods give the urea plus ammonia in urine. If the urea alone is required (as is usually not the case) the ammonia must be estimated by the method given later.

Uric acid. On an ordinary diet the amount of uric acid varies very greatly with the amount of purin in the diet. On a purin free diet the amount passed in 24 hours is fairly constant averaging about 0.4 gram. The chief changes found in the uric acid excretion on a purin free diet are those found in leukaemia in which the amount may be much increased and those found in gout. In chronic gout owing apparently to a specific inability of the kidney to excrete uric acid the amount in 24 hours is persistently low e.g. 0.1 gram. During attacks of acute gout the amount excreted rises to abnormally high figures. Atophan causes an increased excretion both in normal and gouty subjects. In normal persons however this increased excretion cannot be kept up as the body becomes depleted of uric acid.

Uric Acid Estimation

Required. Uranium acetate mixture. In a large flask put 500 grams of ammonium sulphate, 5 grams of uranium acetate and 60 c.c. of 10 per cent acetic acid. Add 650 c.c. of distilled water and shake till dissolved. The volume is almost exactly 1 000 c.c.

10 per cent ammonium sulphate solution

20 potassium permanganate. Dissolve 1.58 grams of pure potassium permanganate in distilled water and make up to 1 000 c.c. in a litre flask. To check the strength of this solution weigh out between 0.1 and 0.2 gram of pure crystalline ammonium oxalate to the nearest milligram. Add about 50 c.c. of distilled water and 3 c.c. of pure concentrated sulphuric acid. Warm on the water bath until dissolved and titrate while hot with the permanganate solution until a faint pink colour appears which lasts for a minute. If V be the

weight of oxalate taken and P the number of cubic centimetres of permanganate required 1 c c of the solution is equivalent to

$$\frac{A}{P \times 0.00355} \text{ c c of } \frac{N}{20} \text{ permanganate}$$

Burette

100, 50 and 25 c c pipettes

500 c c flask and beakers

Funnels, filter papers

Principle (a) Mucin like substances which interfere with subsequent filtration are precipitated from the urine with the uranium acetate mixture (b) The uric acid is precipitated as ammonium urate (c) The ammonium urate is titrated with

$\frac{N}{20}$ potassium permanganate, of which 1 c c = 0.00375 gram of uric acid. A small addition to the figure obtained is made to allow for the solubility of ammonium urate.

Procedure (a) Measure 200 c c of urine into a 500 c c flask, add 50 c c of uranium acetate mixture mix and stand half an hour to let the precipitate settle.

(b) Filter, measure 125 c c of the filtrate into a beaker add 5 c c concentrated ammonia mix and let stand 12 to 24 hours. Decant the supernatant liquid on to a filter 9 cm diam wash the precipitate on to the filter with 10 per cent ammonium sulphate solution and wash on the paper two or three times with the same solution.

Remove the paper from the funnel wash the precipitate into a beaker with a fine stream of water from a wash bottle. Make the water in the beaker up to about 100 c c add 15 c c of strong sulphuric acid and titrate with $\frac{N}{20}$ permanganate until the addition of two drops produces a faint pink flush through the whole fluid.

If x c c of permanganate are used, the uric acid in 100 c c of urine is

$$\left(x \times \frac{A}{P \times 0.00355} \times 0.00375 \right) + 0.003 \text{ gram.}$$

0.003 is the correction for the solubility of ammonium urate

Acid Excretion

In all investigations on the acid excretion in urine the urine must be fresh or have been collected in a bottle containing

toluol or some other preservative. Estimations on 24 hour urines collected in the ordinary way are useless.

The excess of non volatile acids and bases in the blood is removed by the kidneys. If, as is usual, the acid removed exceeds the base, the urine is acid, but a large part of the excess acid is neutralised by ammonia formed in the kidney. The free acid plus ammonia in the urine is equivalent to the excess of the acid over base removed from the blood. When an abnormally large amount of acid is excreted owing to abnormal ingestion or formation, or deficient destruction of acids, three results may occur —

(1) An excess of the particular acid appears in the urine

(2) An excess of free acid plus ammonia appears in the urine unless alkalis are given, in which case the abnormal amount of acid will be excreted as a salt

(3) Retention of the acid in the body may occur, owing to the kidneys failing to excrete the acid sufficiently fast. If alkalis are not given this causes a reduction of plasma bicarbonate (acidosis).

The reaction of urine varies from pH 4.4 to pH 8.6 or more. When large quantities of free acid are being excreted the pH of urine is always low—under 5.5. If sufficient alkali is given under these conditions the pH of the urine rises, but the urine may not become actually alkaline until more alkali has been given than is sufficient to increase the plasma bicarbonate above normal (alkalosis), an undesirable condition. If alkalis are used therefore, it is advisable to give small doses repeatedly (10 grams in 100 c.c. of water every hour) until the urine begins to become definitely more alkaline.

Owing to the small excretion of ammonia in nephritis, with severe renal impairment, the pH of the urine is usually low—about 5.0.

The reaction of the urine may be required in cases in which acid urine, containing ketone bodies is being produced as a method of treatment, in such cases it is only necessary to measure the pH, more than roughly, when the urine becomes acid enough to give a red colour with methyl red.

Estimation of pH

Required (1) A number of test tubes of uniform bore, made of clear, hard glass.

(2) 10 c.c. pipette. Graduated 1 c.c. pipette.

(3) Comparator for holding tubes (Fig 25) This may be made from a wooden box painted dead black inside

(4) 0.2 N acetic acid Prepare from N acetic acid which has been standardised against N sodium hydroxide by diluting 200 c.c. to 1 litre with distilled water

(5) 0.2 N sodium acetate Mix 200 c.c. of N acetic acid with 200 c.c. of N sodium hydroxide and dilute to 1 litre

(6) Methyl red solution 0.2 per cent in 60 per cent alcohol

(7) Brom cresol purple solution 0.04 per cent solution made by diluting 10 c.c. of the stock (1.2 per cent) with 290 c.c. water

(8) Phenol sulphone phthalein solution (0.02 per cent) made

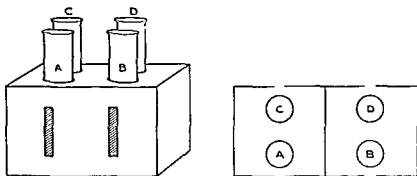


FIG 25 —Comparator

by diluting 10 c.c. of the stock (0.6 per cent) with 290 c.c. water

(9) Series of buffer solutions made by mixing (4) and (5) according to Table VII in the special test tubes (1) and adding 0.2 c.c. of methyl red solution (6) to each. These tubes stoppered with waxed corks may be kept in the ice chest for months

Procedure Filter the urine if it is not perfectly clear. Measure 10 c.c. into one of the tubes. Add 0.2 c.c. of methyl red and mix. If the mixture is yellow without a trace of pink the pH is above 5.8. If not place the tube in position B in the comparator. Run 10 c.c. of urine into another tube and place in position A. In position D put a tube containing distilled water. Into position C put one of the buffer solutions (7). Hold the comparator towards the light (comparisons can only be made by daylight). If the colours as seen through the

slits match, the pH of the urine is the same as that of the buffer mixture. If the urine does not give a pink colour with methyl red, add 0.2 c.c. of brom-cresol purple to 10 c.c. If the mixture is yellow or greenish, the pH is in the neighbourhood of 6, if purple the pH is higher. In the latter case add 0.2 c.c. of phenol sulphone phthalein to 10 c.c. If the colour is yellow the pH is about 7, if red about 8. It is little use investigating the pH on the alkaline side of neutrality as great changes occur after the urine is passed owing to loss of CO_2 .

TABLE VII—*Buffer Solutions prepared by Mixing 0.2 N Acetic Acid (4) with 0.2 N sodium acetate (5)*

pH	(4)	(5)	pH	(4)	(5)
4.6	5.2 c.c.	4.8 c.c.	5.2	2.15 c.c.	7.85 c.c.
4.7	4.6 "	5.4 "	5.3	1.75 "	8.25 "
4.8	4.05 "	5.95 "	5.4	1.5 "	8.5 "
4.9	3.5 "	6.5 "	5.5	1.2 "	8.8 "
5.0	3.0 "	7.0 "	5.6	0.95 "	9.05 "
5.1	2.55 "	7.45 "	5.7	0.75 "	9.25 "

When a large amount of free acid is passed, the urine usually gives a strong red colour with methyl red and alkali administration should be stopped as soon as this colour changes to yellow.

The average amount of free acid passed in 24 hours by normal persons is equivalent to about 300 c.c. of decinormal solution, the range, however, is wide, for the urine may be alkaline and on a high protein diet as much as 700 c.c. may be passed. The estimation of free acid is therefore of little clinical value.

The amount of ammonia in urine of normal persons also varies greatly. On an ordinary diet the amount passed in 24 hours is on an average about 0.5 gram, on a high protein diet the amount may be doubled, but similar reductions do not occur when the protein intake is greatly reduced. When abnormal amounts of acid are being excreted and no alkalis given the ammonia in the urine is increased, and this increase has been used as a measure to the amount of abnormal acid, especially in ketosis, in this condition the amount of ammonia excreted may run closely parallel with the amount of total acetone bodies, but this is not always the case,

and with other acids the parallelism is much less. The method is still used, however, and may be useful in some cases. With moderate increase in the excretion of acids the ammonia may rise to about 1 gram in 24 hours, with greater increase to 2 grams, and in extreme cases to 4 grams. When it is only possible to obtain single specimens of urine, no conclusion can be drawn from the percentage of ammonia in the urine, to correct for variations of concentration in the urine, the ratio of ammonia nitrogen to urea nitrogen may be used instead. Normally, this ratio is about 1 to 20, when it rises to about 1 to 10 the acid excretion is probably abnormally high, in extreme cases the ratio may rise to 1 to 2. This ratio is often used when the whole 24 hours urine is available under the impression that this will correct for the variation of ammonia with protein intake, this, however is not the case for when the protein in the diet falls—a more common event in patients than a high protein intake—the ammonia in the urine does not fall correspondingly and a high ratio is found.

Ammonia may be estimated by two methods—an aeration method which is accurate and a method based on the combination of ammonia with formalin which is rough. Formalin combines with amino acids and other substances in urine, so that the results by the second method are too high.

Aeration Method

Required Apparatus as for urea estimation (p. 76)

10 c c pipettes

$\frac{N}{10}$ hydrochloric acid

$\frac{N}{10}$ sodium hydrate

Anhydrous potassium carbonate

Caprylic alcohol

Methyl red solution

Place 10 c c of urine and 3 drops of caprylic alcohol in tube A. In tube B measure 10 c c of $\frac{N}{10}$ hydrochloric acid.

Add 2 drops of methyl red. Connect up with the pump and run a gentle current of air through the apparatus. Take out the stopper of tube A and drop in 6 grams of anhydrous potassium carbonate, replace stopper, run the air current

gently for 5 minutes then hard for 1 hour. Disconnect, wash entrance tube of B inside and out with distilled water, and titrate the acid with $\frac{N}{10}$ sodium hydrate. If x c c are required 100 c c of the urine contain $10(10 - x) \times 0.0017$ gram of ammonia and if the volume passed in 24 hours is V , the ammonia passed in 24 hours is $\frac{0.0017 \times (10 - x) \times V}{10}$

Formalin Method

Required

2 c c and 20 c c pipettes

10 and 100 c c cylinders

Burette

Two 250 c c conical flasks (A) and (B)

$\frac{N}{10}$ sodium hydrate

Formalin

0.5 per cent phenolphthalein

Principle The urine is neutralised so that ammonia is present as neutral salts. Neutral formalin is then added, this converts ammonia into neutral hexamethylene tetramine, and the acid combined with ammonia is set free. This acid is estimated by titrating with sodium hydrate to the reaction of the fluid before the addition of the formalin. The ammonia in the urine which is equivalent to this acid can then be calculated.

Procedure In each flask measure 20 c c of urine add 80 c c of distilled water and 2 c c of 0.5 per cent phenolphthalein, titrate each with $\frac{N}{10}$ sodium hydrate until the same definite pink colour is obtained in each, cork.

Measure 5 c c of formalin into an 8 × 1 inch test tube add 2 drops of phenolphthalein solution and titrate with $\frac{N}{10}$ sodium hydrate to the first pink colour. Add the formalin to flask (A), the pink colour goes owing to the liberation of acid. Read the sodium hydrate burette and titrate until the colour of (A) is the same as that of (B).

If x c c are used in the last titration the urine contains $5x \times 0.0017$ grams of ammonia in 100 c c.

Acetone Bodies A very small amount of acetone bodies

undetectable by qualitative tests, is normally passed in the urine. With mild degrees of ketosis small quantities of acetone and aceto acetic acid are passed, e g., 0.2 gram of each. With increasing severity of the ketosis β hydroxybutyric acid appears and is excreted in increasing quantities, so that as much as 50 grams in 24 hours may be found. The amounts of acetone and aceto acetic acid do not rise much above 4 grams.

As the amount of acetone bodies passed is some indication of the risk of coma in a diabetic patient, various means have been used to estimate them apart from the somewhat lengthy quantitative method.

The depth of colour obtained with the nitroprusside reaction is not a satisfactory index of the total amount passed, as the depth of colour produced varies greatly with the concentration of other substances in the urine.

The most popular index is the strength of the ferric chloride reaction. As might be expected the results are not very satisfactory, since the substance measured by this reaction is not the principal one excreted when the ketosis is severe. Some idea of its value can be obtained from the following table given by Joslin —

FeCl ₃ reaction	Total acetone bodies reckoned as β hydroxybutyric acid
0	5.7 to 11.0
+	7.0 „ 14.2
++	8.5 „ 55.3
+++	13.3 „ 51.0
++++	17.6 „ 36.8

The most that can be said is that a definite ferric chloride reaction shows that a large amount of acetone bodies (over 7 grams in 24 hours) is being excreted. However the ferric chloride test can be done every day, and with all its drawbacks is probably the most useful measure of the severity of a ketosis.

As an index of retention the nitroprusside test on the blood filtrate (p. 74) may be used whenever blood sugar is estimated. Other methods of investigating acid or ketone body excretion and retention may be employed. Joslin considers that the ketosis is very severe when the total acetone body reaches 30 grams (reckoned as β hydroxybutyric acid) in 24 hours,

when the ammonia in 24 hours reaches 4 grams, or when the plasma bicarbonate is under 30 c c of CO_2 per 100 c c of plasma

Chloride The amount of chloride in the diet, and consequently the amount excreted in 24 hours and the concentration, normally vary greatly The average amount in 24 hours is about 7 grams reckoned as chlorine, or 12 grams reckoned as sodium chloride

Very low concentrations are found in conditions in which the plasma chloride is reduced, such as pneumonia and intestinal obstruction, and occur also in the late stages of nephritis with nitrogen retention, and are characteristic of nephrosis When œdema fluid is being excreted the concentration of chloride in the urine is almost always about 0.6 per cent (as chloride)

Estimation of Chlorides

Chlorides may be estimated in urine by the method used for plasma Measure 5 c c of urine into a test tube, add 10 c c of $\frac{\text{N}}{10}$ silver nitrate solution and 4 c c of nitric acid Heat in a boiling water bath until the silver chloride has formed a coarse precipitate with clear fluid above, cool, add iron alum and titrate with $\frac{\text{N}}{10}$ potassium thiocyanate which should have been checked against the silver nitrate solution as before If potassium thiocyanate equivalent to x c c of silver nitrate solution is required, the urine contains $(10 - x) \times 0.00585 \times 20$ grams of sodium chloride in 100 c c

The amounts advised are usually suitable, if, however, one drop of thiocyanate gives a red colour in the titration, too much urine has been used and the estimation should be repeated, using only 2 c c of urine In this case 100 c c of urine will contain $(10 - x) \times 0.00585 \times 50$ grams of sodium chloride

Diastatic index An enzyme that will digest starch is present in the plasma and is excreted in the urine The amount present in the plasma or urine, the "diastatic index," is measured by the number of cubic centimetres of a 0.1 per cent solution of soluble starch that 1 c c of the fluid under investigation will digest to erythro-dextrin in half an hour at 37°C

The diastatic index of normal urine lies between 6 and 30, that of plasma is slightly less The chief abnormalities are

found in diseases of the pancreas, particularly acute pancreatitis, in which the diastatic index of both plasma and urine may be much increased, up to 200 or more, and in kidney diseases, with gross impairment of renal efficiency, in which the diastatic index in the urine is reduced without corresponding reduction in the index of the plasma

Estimation

Required 0.1 per cent soluble starch Weigh out 1 gram of soluble starch rub up with cold water and pour into boiling water stirring meanwhile Cool and make up to 100 c c Keep this stock solution in an ice chest For use dilute 5 c c to 50 c c with normal saline This 0.1 per cent solution should be made up fresh each day

Iodine solution made by diluting a 4 per cent solution about twenty times with water

Series of test tubes $3 \times \frac{1}{2}$ inch

Three graduated 2 c c pipettes two graduated 10 c c pipettes

Procedure Collect the whole 24 hours urine in a Winchester quart bottle under toluol Before use shake up the precipitate as the enzyme may be partly adsorbed by this

Measure 8 c c of normal saline into a test tube add 2 c c of urine and mix well Into a series of test tubes measure this mixture normal saline and starch solution according to the table

	Diluted urine	Saline	Starch solution	Diastatic index
A	2 c c	0	1 c c	25
B	1.5	0.5		33
C	1.2	0.4	"	42
D	1.0 ,	1.0		50
E	0.75 ,	1.25	"	67
F	0.50	1.50	,	100
G	0.30	1.70	,	167
H	0.20 ,	1.80	,	250
J	0.10 ,	1.90	,	500

The urine mixture and saline should be added first to all the tubes, and then the starch added to the series rapidly Put

the tubes at once in an incubator, or better a water bath, at 37°C . After half an hour remove from the incubator or water bath, and at once add to each tube 2 drops of the iodine solution. Note the tube with the least quantity of urine that does not give a purplish colour, this contains the least amount of urine that will digest 1 c.c. of 0.1 per cent starch to erythro-dextrin. The corresponding diastatic index is given in the table. If the urine is concentrated, the iodine may be used up in oxidising the urine in the first tubes of the series, in this case add 2 more drops.

If the starch in all the tubes is digested dilute 1 c.c. of the mixture of urine and saline with a further 9 c.c. of the saline and test again using this further diluted mixture with the proportions as in tubes E to J, the corresponding diastatic indices will be ten times as great. If a high index is expected this second series should be put up without waiting for the results of the first series.

Some variation in the index is produced by variation of the pH but the changes so produced are not sufficiently large to be of practical importance in the clinical application of the test.

DETECTION OF LEAD

Principle The lead in the urine is carried down together with a precipitate of earthy phosphates. The precipitate is ashed and redissolved. A small amount of copper solution is added. On the addition of H_2S the lead is carried down with the copper sulphide formed, and detected by the formation of crystals of lead hexanitrite.

Required

- (1) Saturated solution of ammonium sulphate
- (2) 2 per cent solution of copper acetate
- (3) Sulphuretted hydrogen
- (4) 4 per cent sodium acetate solution
- (5) 10 per cent acetic acid solution
- (6) Potassium nitrate
- (7) Capillary tube, two graduated 0.1 c.c. pipettes

Procedure Collect the urine for 24 hours, preserving with toluol. Pool the urine in a large flask and add sufficient strong ammonia solution to make strongly alkaline. Stand overnight. Decant as much as possible of the supernatant fluid and filter off the precipitate on a Buchner funnel. Ash the precipitate in a silica dish until white. Dissolve the ash in

hydrochloric acid and make the volume up to about 25 c c. Neutralise with sodium hydroxide solution and make just acid to methyl orange with dilute hydrochloric acid. Add 1 c c of saturated ammonium sulphate solution and 1 drop of copper acetate solution and saturate in the cold with sulphuretted hydrogen. Centrifuge and decant the supernatant fluid. Wash three times with distilled water centrifuging and decanting the supernatant fluid. It is essential to drain off the supernatant fluid thoroughly each time. Place the centrifuge tube containing the precipitate in a beaker of boiling water and add 2 drops of nitric acid. Draw up a drop of the solution thus obtained in a capillary tube and evaporate it on a slide. Add 0.02 c c of sodium acetate solution completely dissolving the residue. Evaporate again aiming at obtaining the residue in a ring about 0.4 cm. in diameter. Chill the slide on ice. Add 0.01 c c of 10 per cent acetic acid and place a small crystal of potassium nitrite in the centre of the ring. The potassium nitrite diffuses slowly to the margin and if lead is present crystals of lead hexanitrite are found as black cubes and squares visible under the microscope among the crystals of copper acetate.

The urine should not be allowed to become alkaline and deposit phosphates spontaneously as in this case the lead may not be carried down nor is it satisfactory to redissolve the precipitate with acid or reprecipitate with ammonia.

CHAPTER XVII

URINARY CALCULI—BACTERIOLOGY OF URINARY TRACT

THE calculi which may be found in any part of the urinary tract can be divided into those which are comparatively common and those which are extremely rare. We are only concerned here with the composition of the various calculi and this is readily determined by a short and simple scheme of analysis. The exact percentage composition of mixed calculi is naturally a more laborious proceeding but does not form a part of ordinary clinical pathology.

Speaking generally, calculi may vary in size from a small concretion such as may be passed by the urethra, to a mass the size of a clenched fist. They are commonly pigmented. They may be smooth or rugged. They tend to take the shape of the viscus in which they have been formed, as, for example, the pelvis of the kidney. If multiple they may be faceted. The fractured surface frequently shows concentric rings. Calculi are commonly of mixed composition, and the nucleus may be formed of a different material to the cortical portion. The comparatively common stones are calcium oxalate, uric acid, ammonium urate and phosphatic calculi.

Calcium oxalate stones mixed with a certain amount of calcium phosphate would appear to be the most common variety. Calcium oxalate calculi are as a rule hard, of a reddish brown colour, and with a granular surface which has given them the name of "mulberry" calculi. They may be branched.

Calcium phosphate calculi are common especially in the bladder, they are found particularly when inflammation is present. Layers of phosphate may form on the surface of foreign bodies or on stones composed of other substances. A small amount of calcium carbonate may be mixed with the phosphate. These stones are usually white and crumbling.

Uric acid calculi are less common and contrary to the statements of many text books are extremely rarely found in

the kidney, though they are not very infrequently met with in the bladder. The rarity of uric acid calculi in the kidney is fortunate, since the permeability of uric acid to the X rays is the same as that of the belly wall, consequently they cannot be recognised by X-ray examination. The calculi are usually small, chocolate coloured, fairly hard and have a smooth surface.

While pure uric acid calculi are rare uric acid is fairly commonly found in association with some other substance such as calcium phosphate or oxalate. The mixed calculus can be recognised by X ray photography.

Urate stones consisting of ammonium, with a lesser amount of sodium urate are hard stones of a brown colour.

The composition of the commoner calculi should be sought according to the following scheme of examination. If the *reactions for those substances are not given, or only traces of* them are found, some of the rarer substances must be tested for, as subsequently described.

Chemical Examination

If the stone is of considerable size, cut it in half

Examine the cut surface, and if the nucleus differs in appearance from the cortical portion, scrape out the nucleus first and examine it separately.

Powder up the calculus in a clean mortar.

The principal constituents of the stone may then be identified as follows —

To a knife point of the powder, in a test tube, add about 5 c.c. of dilute hydrochloric acid. If effervescence occurs carbonates are present. Heat. If the powder does not dissolve test the powder for uric acid*. If the powder dissolves cool and add strong ammonia until alkaline. If no precipitate appears test for cystine and xanthine. If a precipitate appears the stone contains oxalate or phosphate. Add acetic acid until acid. If the precipitate does not dissolve the powder contained calcium oxalate, if it dissolves the powder contained phosphate. The presence of phosphate may be confirmed by dissolving a knife point of the powder in strong nitric acid, adding excess of a 10 per cent solution of ammonium molybdate and boiling. A heavy yellow precipitate indicates the presence of phosphates.

* Solution is usually not complete with any stones owing to the presence of blood pigment, etc.

To test for *uric acid* add 3 drops of strong nitric acid to a knife point of powder in a porcelain dish and evaporate to complete dryness on a water bath, if uric acid is present a reddish colour appears, which turns violet (murexide reaction) on adding dilute ammonia (5 drops of strong solution to a test tube full of water) To determine whether the uric acid is present as ammonium urate place some powder in a test tube add about 2 c c of strong sodium hydroxide and heat Test for the evolution of ammonia by smell and with a piece of moist litmus paper held at the mouth of the tube If ammonia is detected the stone contained ammonium urates, if, as is more usual no ammonia is present, the material was uric acid

To test for *cystine* dissolve a knife point of the powder in about 3 c c of strong (10 per cent) sodium hydroxide solution add a few drops of lead acetate solution and boil If cystine is present the fluid turns black As a confirmatory test the powder may be dissolved in strong ammonia solution A few drops are placed in a watch glass and the ammonia allowed to evaporate Typical hexagonal plates separate out If a drop of strong hydrochloric acid is allowed to flow over the deposit, as each crystal is bathed in the acid there springs from it a stellate cluster of prisms

Xanthine stones are very rarely found They are hard and dissolve readily in alkalines or ammonia, slowly on warming with hydrochloric acid They may be recognised by a reaction similar to the murexide test, on heating with nitric acid a lemon yellow colour is given which changes to orange on the addition of 10 per cent sodium hydroxide and to purple red on further heating

Concretions, soluble in ether and consisting mainly of cholesterol, have been described (urostealiths) The cholesterol may be recognised by the methods described under gall stones

THE BACTERIOLOGY OF THE URINO-GENITAL TRACT

The variety of pathogenic organisms commonly met with in the urino genital tract is not very great, and in general the bacteriological examinations should be conducted on the lines indicated in previous sections The *gonococcus* is described on p 99, and the methods for isolating it from the genito urinary

tract on p 231 An account of the *S pallida* and the means of detecting it on pp 131 and 229

Ducrey's bacillus is described on p 115

The tubercle bacillus In examining the urine for tubercle bacilli (see p 227), particular care should be taken with ureteric specimens, the deposits of which should be examined for pus as well as for the bacilli These specimens almost invariably contain red blood cells resulting from the passage of the catheter Pus cells must not be confused with the small round, or elongated mononuclear epithelial cells, which are normal constituents of ureteric specimens The amount of albumin, in the absence of gross contamination by blood, and the percentage of urea should also be determined in suitable specimens In a tuberculous case in which pus and tubercle bacilli are present in the bladder and in one of the ureteric specimens, failure to detect tubercle bacilli or pus cells in the other ureteric specimen is of the greatest assistance in deciding the advisability of nephrectomy

The colon bacillus A catheter specimen, carefully taken after cleaning the urethral orifice, is essential for the detection of colon bacilli in the female In the male a catheter specimen is not essential The glans penis should be thoroughly cleansed, and the patient should be instructed to pass the first portion of urine into an ordinary receiver and the next portion into a sterile wide mouthed flask fitted with a wool plug

When the specimen is obtained proceed as follows —

Pour approximately 3 to 4 c c of urine into a broth tube Set aside the remainder of the specimen for further investigation

Incubate the culture at 37° C for from 12 to 24 hours

Examine the broth culture for general turbidity, and microscopically for the presence of bacilli

If a growth of bacilli has taken place, prepare two Petri dish plate cultures, one of which contains agar and the other MacConkey's medium

Take a platinum loop of the broth culture and make a series of streaks, first on the agar plate and next (without recharging the loop) on the MacConkey plate

Incubate the plate cultures and the original broth culture till the next morning

Examine both plates and in particular the MacConkey plate If large red colonies are present on the MacConkey

plate and all are apparently of the same nature subculture from them into the following media Litmus milk Neutral red broth Broth Gelatin slope Litmus dextrose Litmus mannite Litmus lactose Incubate the sub-cultures until the next morning, but remember to place the gelatin slope in a separate crate at a temperature of from 18° to 22° C

Examine the sub-cultures for the following changes —

Acid and clot in milk, green fluorescence in neutral red broth, indole in broth, as shown by the production of a rose pink colour on the addition of nitric acid, growth on gelatin without liquefaction, acidity and gas formation in the three litmus carbohydrate media

If all these changes are present the organism is the colon bacillus

If the changes have not yet occurred, reincubate the tubes for another 24 or 48 hours, or longer if necessary The indole reaction frequently takes a week to develop

The agar plate should also be examined for the presence of cocci in addition to the bacilli Colonies of staphylococcal or streptococcal nature should be examined microscopically, and if cocci are present sub-cultures should be made in broth or on agar and the cultural character of the organisms further investigated

No cocci will be found on the MacConkey plate

If bacilli of the colon type are found on the agar plate and *no growth has taken place on the MacConkey plate this should be reinoculated from the original broth culture* If no growth occurs after reinoculation, colon bacilli are absent

If colonies of more than one type or colour are present on the MacConkey plate, a characteristic discrete colony of each type must be transferred to broth and sub-cultures from each broth tube made on the following day into the series of media given for the colon bacillus

The catheter specimen of urine must on every occasion be examined generally as well as bacteriologically Very little information is derived from the cultivation of a colon bacillus from the urine, and such information as is given may be totally misleading Colon bacilli in the urine are not by any means the necessary explanation of an obscure fever or even of definite urinary symptoms All bacterial investigations must be considered in conjunction with the general examination of the urine and the clinical condition of the patient The further

examinations of the urine to be invariably made are simple, and concerned with the appearance, the reaction, the presence and amount of albumin, and the nature of the deposit

The following types of infection may be recognised —

In the *latent type* colon bacilli may exist in the urine without producing any signs or symptoms of disease. In such cases the urine is of normal appearance, no bacilli are seen in the centrifuged deposit, and a growth of the colon bacillus is obtained on culture. This class of case constitutes the "colon carrier." The condition is liable to revert to the acute inflammatory stage, particularly if pregnancy or some other complication arises.

In the *acute type* the patient has marked vesical and often renal symptoms, has high fever sometimes with rigors and may present all the aspects of extremely serious illness. The urine is turbid on passing the turbidity being due to the enormous numbers of bacilli. Pus cells are present, but they may be scanty and red cells may be found. A trace of albumin is present. Such patients practically always recover from this severe stage under treatment and often, apparently, in spite of treatment. They, however, frequently pass into the chronic type.

In the *chronic type* which may persist for months and may be apparently chronic from the onset, some other predisposing cause, such as pregnancy, obstinate constipation, hæmorrhoids, or an ischio rectal abscess, is often present. The urinary symptoms are subacute or absent, a mild and intermittent pyrexia is present, and the urine is acid containing a trace of albumin and a considerable naked-eye deposit due to pus and bacilli.

The presence of colon bacilli, therefore, in a culture taken from the urine is insufficient evidence that the bacilli are actively producing disease, and if no pus is present it is almost certain that they are not. Even if pus and bacilli are present it does not follow that the ultimate diagnosis has been reached. Infection by the colon bacillus is readily superimposed upon some other lesion, such as that of tuberculosis or calculus. The *bacillus proteus* is in such instances often present in addition.

In all cases of coli infection of the urine associated with a considerable degree of pyuria it is a wise precaution to search the urine for tubercle bacilli also.

The treatment of coli infections by autogenous vaccines is worthy of trial in selected cases

The bacillus proteus The examination for this organism is conducted in the same manner as that for the colon bacillus. The bacilli grow well on the MacConkey plate and are recognised by the yellow colour of their colonies the more slimy nature of their growth and their somewhat characteristic and offensive odour. The cultural character which mainly distinguishes them from the colon bacillus is the power of liquefying gelatin. Pure infections of the urinary tract by *B. proteus* occur but the organism is more frequently found in association with other bacteria and other lesions of the tract such as neoplasm of the bladder or renal calculus. In cases of proteus infection the urine is commonly alkaline.

The typhoid bacillus This organism is examined for by the same routine. The cultural characters are given under the description of the bacillus. The bacillus should be further identified by serum agglutination tests using both the serum of the patient and a stock anti-serum.

The typhoid bacillus may exist in the urine without producing symptoms and in such cases is a dangerous source of infection to others.

Bacilluria following typhoid fever is common but is in the great majority of cases due to the *B. coli*.

Staphylococci, Streptococci, Diplococci. Any of the ordinary pyogenic cocci may be found in the urine but in the majority of cases they come from the lower part of the tract and are of little importance.

The specimen for examination must be a catheter specimen and preferably should be withdrawn after thorough irrigation of the urethra with sterile water.

The urine should be first added to broth and the centrifuged deposit examined as soon as possible. If cocci are numerous in the recently passed specimen they must have been present in the bladder before passage. Cocci in a specimen that has been standing overnight may be urethral contaminants. The broth culture is subsequently plated in the usual way. Any of the three varieties of staphylococci may be present and may produce a cystitis associated with a purulent and often alkaline urine. Staphylococcal cystitis is rarely primary and is more often superimposed upon some other lesion such as a urethral stricture or prostatic enlargement. In staphylococcal

pyæmia the causative organism is usually abundant in the urine

Streptococci are less frequently met with if the urethral lavage has been thorough. A long chained streptococcus of low pathogenicity to animals is the variety most commonly found. Hæmolytic streptococci are sometimes present and may be associated with a prostatitis or urethritis.

Pneumococci in the urine are often recorded and rarely found. The organism commonly confounded with the pneumococcus is the *enterococcus* a paired coccus met with frequently in the urinary tract. This diplococcus is Gram positive and morphologically resembles the pneumococcus but is readily distinguished by its cultural characters. The organism is more commonly met with in specimens from women and children than from men and in the majority of cases gives rise to no symptoms. It may however be found in pure culture in cases of cystitis or pyelitis and it has been recovered from the general circulation.

Other organisms *B. melitensis* can in the majority of cases of Malta fever be isolated from the urine during the early period of the disease. Bacilli of the *influenza* type are occasionally met with in association with a purulent urethral discharge in which no gonococci can be found and in rare cases neither gonococci influenza bacilli nor any other bacteria can be detected in films or cultures of the pus. Such unusual infections may follow sexual intercourse.

Diphtheroid bacilli are frequently found in the urine and are practically always derived from the lower urethra. The appearance of these organisms in the cultures is evidence of urethral contamination. The bacilli are apparently harmless.

In conclusion any of the organisms of the colon typhoid group not previously mentioned may be found in the urine and bacilli of this group which are difficult to classify exactly are fairly frequently met with.

CHAPTER XVIII

RENAL EFFICIENCY TESTS

It should be explained that there are, from the chemical point of view at any rate, two processes which occur in nephritis. The first often called glomerulo nephritis, in which frank inflammatory changes occur, leads to impairment of renal efficiency as measured by the tests here discussed. The second process, nephrosis, characterised microscopically by degeneration of the tubules and clinically by œdema, does not lead to such impairment, when, as not infrequently happens, it occurs without glomerulo nephritis, in other words, cases occur with gross œdema, and much albumin and casts in the urine, in which these tests will show no impairment even after many months, in such cases no inflammatory changes are found microscopically. Impairment of renal efficiency is also associated with kidney damage resulting from other forms of inflammation, from hyperpiesia, or from congenital cystic disease.

Blood Urea

Of the many renal efficiency tests that have been proposed by far the most useful is the simple estimation of the *blood urea*. It is true that high values for blood urea may be found in other conditions, but if, as is usually not difficult, these conditions are excluded, a high blood urea may be taken to mean that the kidneys are so impaired that they can no longer keep pace with their daily task of excreting urea. The blood urea may be altered experimentally by changes in the nitrogen intake, and it might be expected that the diet would have to be considered in weighing the significance of blood urea findings. In actual practice the variations of diet do not seem adequate to introduce appreciable errors. In a long series of cases, in which microscopic examination of the kidney became possible during life or after death, we have found that the height of the blood urea gave a good indication of the degree of damage of the first type (glomerulo nephritis) that the kidney had suffered

The blood urea does not rise, however, until a certain degree of damage has occurred

Prognosis in chronic medical cases. The statement that patients whose blood urea is over 0.1 gram per 100 c.c. have not more than a year to live seems to be fairly correct. Patients may live for some years with blood ureas of 0.070 or 0.080 per cent, but the figures begin to rise to about 0.1 only when the kidneys are finally failing. The higher the figures go the shorter the prospect of life so that when they rise above 0.3 per cent only a few weeks of life may be expected. It is rare for the blood urea to rise above 0.6 per cent. These are not unvarying rules but statements of probability. Patients with congenital cystic kidneys are exceptional; they may live a considerable time and be moderately active with very high blood ureas.

In acute nephritis great increase of the blood urea is unusual, although the prognosis is more serious when the blood urea rises above 0.1 per cent, complete recovery is possible.

In chronic cases with œdema the blood urea gives a measure of the degree of damage due to glomerulo nephritis associated with the nephrosis. This is of practical interest, for whereas complete recovery from pure nephrosis, although rare, may occur, complete recovery from the changes produced by chronic glomerulo nephritis is impossible.

It has been maintained that inorganic phosphate and creatinin are of more value for prognosis than the blood urea, as they only rise to very high values shortly before death. In our experience, however, the inorganic phosphate is less satisfactory than the blood urea, and in any case it is the more distant prognosis which is of value, for usually when death is near, its imminence is obvious from clinical examination.

Prognosis for operation in prostatic hypertrophy. Unsatisfactory results whether directly due to kidney damage or to other causes, are much more common among patients whose blood urea is above 0.050 per cent, and are rare among those with blood urea below 0.040 per cent. Patients whose blood urea has fallen from a high to a normal figure after drainage are still apt to do badly.

Hyperpiesia. The blood urea in hyperpiesia may remain normal for several years, and figures over 0.1 per cent are not common even at the end. This provides a useful distinction from nephritis with high blood pressure, for high blood

pressure secondary to nephritis is always associated with high blood urea

Functional albuminuria The blood urea is normal this however is no help in diagnosis as normal or low figures are found in the types of nephritis with which it may be confused Nor do other renal efficiency tests give any more help

Uræmia This term is certainly used for three conditions (1) The nervous manifestations that occur in hyperpæmia (2) Sthenic or Pseudo uræmia characterised by convulsions and coma (3) Asthenic or True uræmia characterised by drowsiness and twitching The second type occurs in patients whose kidneys are not grossly impaired the blood urea is usually raised but not very high The third type occurs terminally in patients whose kidneys are completely failing the blood urea is very high inorganic phosphates are high and plasma calcium low It is possible that the low calcium is the cause of the twitching

Other renal efficiency tests Since some degree of renal impairment may be present although the blood urea is normal and a high blood urea due to a temporary interference with excretion may persist for some days after the interference has been removed other tests are required Of the many that have been proposed we will describe three MacLean's urea concentration test the urea clearance test and phenolsulphonephthalein excretion

MacLean's Urea Concentration Test

Renal impairment shows itself first in reduction of the power of the kidney to excrete urine containing a high concentration of urea The object of this test is to measure this power As a stimulus to the kidney a dose of urea is given by the mouth The patient empties his bladder and drinks 10 grams of urea dissolved in 100 c.c. of water and flavoured with lemon With children the dose of urea should be reduced to 12 grams between 8 and 12 years 10 grams between 5 and 8 years 7.5 grams between 3 and 5 years 6 grams between 1 and 3 years and 4 grams under 1 year The urine passed in each of the subsequent 3 hours is collected measured and the urea concentration estimated The urine passed by normal persons usually contains at least 2.5 per cent of urea but if the volume of urine passed in an hour is large (over 120 c.c.) as is not

unusual in the first hour even when precautions to reduce water output are taken the concentration may be below this. Patients who excrete 2.5 per cent or a higher concentration of urea have little kidney damage.

In persons with moderately damaged kidneys values between 1.5 and 2.5 per cent are usually found while in patients with more severely damaged kidneys the concentrations fall below 1.5 per cent. Patients with chronically raised blood ureas are almost always found in this last group. The volume must be taken into account to some extent and it is not safe to draw conclusions from low concentrations when the volume of the urine passed in an hour exceeds 120 c.c. As a large volume is frequently passed in the first hour it is usually advisable to estimate urea only in the second and third hours.

A wide range of concentration may be expected in persons with moderately damaged kidneys as with normal persons, so that the concentration found is not an exact measure of the efficiency of the kidneys in this group *e.g.* it does not necessarily follow that a person who passes 2.2 per cent of urea has better kidneys than one who only passes 1.7 per cent. When however we come to the severely damaged group there is more certainty that the kidney is doing its best all the time and the concentration obtained more fairly measures the efficiency of the kidney.

When the blood urea is already high it is not necessary to give a dose of urea.

It is preferable to perform this test the first thing in the morning when the patient has had nothing to drink since the evening. This reduces the risk of large volumes of urine which may render the result valueless.

This test is obviously rough as no account is taken of the level of the blood urea, and little account of the volume of urine passed.

Harrison's test avoids the first objection. The blood urea is estimated during the third hour after the administration of urea and the ratio milligrams urea in the urine to milligrams urea in the blood is calculated. Normally this should lie between 40 and 70.

Urea Clearance Test

In this test a formula is used which corrects both for variations of blood urea and urine volume. If the rate of excretion of urine is above a certain level (about 2 c.c. per

minute) the relation between urea concentration in the blood B , urea concentration in the urine U , and volume of urine per minute V , is given by the formula

$$\frac{UV}{B} = \text{maximum clearance} = C_m \text{ (a constant)}$$

If, however, the rate of excretion of urine is lower the relation is given by

$$\frac{U\sqrt{V}}{B} = \text{standard clearance} = C_s \text{ (a constant)}$$

Since these formulæ appear to be very accurate for both normal and diseased persons, and since the constants do not vary greatly from person to person, a method based on these formulæ should provide an accurate method of measuring the ability to excrete urea.

The average value of the maximum clearance found in normal persons is 75, and that of the standard clearance about 50. Hence the maximum clearance can be calculated as per cent of the normal by the formula $\frac{UV}{B} \times \frac{100}{75}$, and the standard

clearance by the formula $\frac{U\sqrt{V}}{B} \times \frac{100}{50}$.

In practice the patient empties his bladder and the time is noted. He is allowed $\frac{1}{2}$ pint of water to drink and is kept at rest without food or further drink for 2 hours. All the urine passed in the next 2 hours is collected in two separate bottles, the volumes of the two samples are measured and the urea concentrations estimated. Blood is taken for urea estimation at the end of the first hour. The clearance is calculated for each hour as a percentage of the normal using the maximum clearance formula if the volume was over 2 c.c. per minute and the standard clearance formula if the volume was below this level. Values between 75 and 50 per cent of normal should be considered doubtful. Figures below 50 imply renal deficiency. The test proves more delicate than the phenol sulphonephthalein test but unless the co-operation of the patient and the nursing staff are satisfactory the results are apt to be spoiled by incorrect collection of the hourly urines.

Phenolsulphonephthalein Excretion

This dye is excreted almost solely by the kidneys and its excretion is almost independent of the rate of water excretion.

Required Ampoules containing a solution of phenol sulphonephthalein 6 mgm in 1 c c Weigh out 0.6 gram of phenolsulphonephthalein, rub up in a mortar with 17 c c of $\frac{N}{10}$ NaOH Pour into a 100 c c flask, add $\frac{N}{10}$ HCl till the fluid is orange make up to 100 c c with normal saline and sterilise rapidly Quantities slightly exceeding 1 c c should be measured into sterile ampoules

40 per cent sodium hydrate

Standard phenolsulphonephthalein solutions Dilute 6 mgm of phenolsulphonephthalein dissolved in 1 c c to 100 c c with water made slightly acid with HCl This stock solution keeps well in the dark Take 10 c c of this solution make alkaline with sodium hydrate solution and dilute to 100 c c (1) with distilled water giving Standard I (2) with a concentrated urine giving Standard II These standard solutions should be made up duly as phenolsulphonephthalein is slowly destroyed in alkaline solution

Funnels and filter papers

Colorimeter

Measuring cylinders

Procedure Inject 1 c c of the phenolsulphonephthalein solution (= 6 mgm of dye) intramuscularly and collect separately the whole of the urine passed in each of the two following hours Estimate the amount of dye in each of these urines as follows Make strongly alkaline with sodium hydrate solution make up to a convenient volume of about the same depth of colour as the standards mix well and filter Make a mixture of Standards I and II to contain about the same proportion of red colour from the dye and yellow colour from the urine as the unknown Compare in the colorimeter with this standard at 20 mm if the reading of the unknown is x , and the volume to which the hour's urine was diluted is V , the amount of dye contained in this hour's urine is $\frac{20}{x} \times \frac{V}{10}$ per cent

of the original dose of 6 mgm It is necessary to have urine in the standard otherwise owing to the yellow colour of the urine in the unknown it would be impossible to obtain a match

If the Myers colorimeter is used pour the mixed standard into the left tube and the unknown into the right hand tube each up to the mark 10, then dilute the stronger, adding $\frac{1}{2}$ c c of water at a time, until a match is obtained If the

reading of the unknown is x and that of the standard y , the hours urine (volume V) contains $\frac{x}{y} \times \frac{V}{10}$ per cent of the original dose of 6 mgm

Normal persons excrete over 60 per cent of the dose (that is over 3.6 mgm) in 2 hours, the greater part in the first hour. In patients an excretion of 50 per cent or more in 2 hours shows that no appreciable kidney damage has taken place. With moderate degrees of reduction of renal efficiency values down to 30 per cent in the 2 hours are found and with more severe degrees under 30 per cent. In patients whose kidneys are finally failing very small amounts and even no perceptible trace may be excreted.

When cystitis is present the phenolsulphonephthalein found in the 2 hours' urine is often much less than would be expected from the state of the kidneys, in such cases little significance can be attached to low figures.

With both phenolsulphonephthalein and urea concentration tests it may be said that when results are either normal or undoubtedly bad definite conclusions can be drawn. Intermediate results give an indication of the probable state of the kidney but there is a great deal of individual variation.

The diastatic index of the 24 hours' urine may be used as an index of renal efficiency, it is claimed that when the index falls below 3 the kidneys are severely damaged. We have not found the test useful.

SECTION V—THE ALIMENTARY SYSTEM

CHAPTER XIX

THE MOUTH—THE STOMACH

THE MOUTH

THE laboratory investigations of the buccal cavity are mainly bacteriological in nature

Oral sepsis and intestinal toxæmia have borne the odium of all the ills which man may suffer. There can be no question that a thoroughly septic condition of the mouth leading to an absorption of offensive pus may set up considerable disturbance of which the most direct effect is gastric or intestinal in nature. More remote lesions due to the absorption of toxins into the circulation are also possible but difficult to prove. Of these the most probable is a variety of osteo arthritis which more particularly attacks the phalanges and is very commonly associated with some local septic lesion such as pyorrhœa alveolaris. The bacteriological investigation of the mouth is reasonable in such cases and is occasionally profitable. The practitioner should however be warned against the indiscriminate use of vaccines prepared from organisms of the buccal cavity. Nothing is more simple than the preparation of such a vaccine for any and every condition and nothing would be more futile were it not for the mental effect of a hypodermic injection upon a confiding patient.

Pyorrhœa alveolaris is readily recognised by the exudation of pus from the sockets of the teeth. In severe cases the majority of the teeth are loose and almost floating in a purulent bed. The teeth themselves may show little or no signs of caries. In order to make a cultural examination the mouth should be well washed out with clean and preferably sterile water and a loop of pus as it exudes after pressure on the crown of a tooth be taken in a platinum wire. If little discharge is present

a sterile wool swab similar to that used for diphtheria cases may be rubbed against the root of the tooth. If it is advisable to extract a tooth cultures may be taken from the socket. The platinum wire or swab should be rubbed over the surfaces of 3 or 4 agar or serum agar slope or preferably by a series of streaks over 2 blood agar plates. Films of the pus should also be made. The cultures are incubated till the next morning and the nature of the colonies investigated by the hand glass and microscopically. Sub-cultures should be made from each variety of colony on the plate and their full cultural characters investigated. The predominant organism in the pus films and in the plate cultures should be noted. In the films of pus numerous spirilla and fusiform or beaded bacilli can often be seen but they do not grow in the cultures. If a vaccine is required it should be made from the predominant organism of greatest virulence and in a case of doubt a mixed vaccine can be prepared. The bacteria most commonly found and most frequently predominant in the plate cultures are streptococci either of the viridans type or of the hemolytic varieties. Other organisms which may be obtained are micrococcus catarrhalis and less commonly the pneumococcus. Staphylococci are usually present in addition and almost any of the pathogenic organisms including the bacillus coli may occasionally be isolated.

Thrush is an infection of the buccal cavity by the *oidium albicans*. It is common in children but occurs also in adults and particularly in febrile patients the care of whose mouths has been neglected. A white flaky membrane is present and may spread over the entire buccal cavity including the palate and the tongue. The membrane is usually detachable without leaving a raw surface. In children the membrane has to be distinguished from particles of milk left in the mouth after feeding. In films made from the membrane an abundant interlacing mycelium is usually found together with a few of the yeast like oval cells. In cultures the cellular element of the organism may predominate.

The condition is readily cured by ordinary antiseptic treatment.

Diphtheria produces a membrane which is most commonly confined to one tonsil and is removed with difficulty leaving a bleeding surface. There may be little constitutional disturbance. The methods of taking and examining swabs and

cultures from suspected cases of faucial diphtheria have been already described (p 107)

Vincent's angina consists in an ulcerative stomatitis often with membrane formation. The condition is most frequently met with in young ill nourished children and is accompanied by severe general symptoms. In films made from the ulcerated surface the organisms associated with Vincent's name are very numerous. Pus cells may be scanty and lying on a background of innumerable spirilla of varying shape and size. The fusiform bacilli are less numerous and of similarly variable size some being stout and cigar shaped others long thin and beaded. It is by no means certain that these organisms are the essential cause of the stomatitis. They are very numerous in almost any septic mouth and in Vincent's angina other pyogenic organisms such as streptococci and staphylococci are present in addition.

Follicular tonsilitis is characterised by swelling of one or both tonsils and by numerous small round yellow spots of suppuration in them. The organisms found in the pus are commonly staphylococci or streptococci. Streptococcal inflammation may produce swelling and hyperæmia of the tonsil only and these organisms are usually associated with the angina of scarlet fever as well as with the tonsilitis which almost invariably precedes by 10 days or a fortnight an attack of rheumatic fever. The streptococcus associated with scarlet fever has been previously described (p 96). Streptococci may also set up a membranous tonsilitis closely resembling that of diphtheria but usually associated with severer constitutional disturbance. The distinction between the two conditions is readily made on bacteriological grounds.

Saliva The variations of mucin and diastase in saliva in disease have been little studied.

Estimation of *urea* in saliva by the method given for blood gives concentrations about three quarters of the concentrations found in blood. a variable amount of the urea is converted into ammonia in the mouth but this change does not affect the apparent urea concentration. An estimation of urea in the saliva may be used as a rough method of detecting great nitrogen retention when blood cannot be obtained. The large amount of ammonia formed in the saliva when the blood urea is high gives an ammoniacal smell to the patient's breath.

THE STOMACH

Laboratory investigations into the functions of the stomach are mainly chemical in nature and are principally concerned with an analysis of the gastric juice. The diagnosis of a condition associated with gastric symptoms as dyspepsia has little real meaning and carries us no further than a diagnosis of anæmia in other conditions. A careful analysis of the gastric contents associated with the clinical examination of the patient enables us in the great majority of cases to recognise the condition present and the treatment which should be adopted. Of all the methods of investigation into the composition of the gastric juice that of the test meal analysis is the most important.

The Test Meal The sole difficulty in the procedure lies in the withdrawal of the test meal in reality a very simple *manœuvre*. The passage of an œsophageal tube is uncomfortable but free from pain or danger in careful hands. The objection of the patient to its passage varies inversely with the tact and confidence of the practitioner.

The meaning of the results obtained by the analysis of gastric contents will be discussed later. If the various precautions indicated below are properly observed the results of the analysis may be depended upon. It must be very clearly understood however that these results considered alone are of practically no value in diagnosis but must be read in conjunction with the signs and symptoms of the patient. It is a most elementary error to suppose that absence of free hydrochloric acid in a test meal means that carcinoma of the stomach is present. There are even circumstances in which absence of free acid is opposed to the diagnosis of carcinoma. A carefully taken history of the case read in conjunction with the analysis of the test meal will usually lead to a correct diagnosis but all other methods of investigation should be made use of.

The following is the technique of gastric analysis —

(1) *Lavage of the stomach* It is not necessary to wash out the stomach before giving the meal in the majority of cases and indeed a more reliable result is obtained if lavage is omitted. In cases of obvious dilatation of the stomach with retention of food the stomach should be emptied and washed out with water a few hours before giving the meal.

(2) The *test meal* should be of a simple character. It is most important that in any series of observations the same

type of meal should be given, and that in any single observation the nature of the normal result obtained from such a meal should be known. The figures given here are those obtained from gastric contents after a test meal which is practically that of Ewald.

The meal consists of two large cups of tea, with milk and sugar if desired, and two rounds of toast lightly buttered.

The actual bulk of the meal is unimportant since the quantity of gastric juice accommodates itself to the bulk of material in the stomach. The quantity given however should be considerable in order to facilitate the subsequent withdrawal.

(3) *Removal of the gastric contents* These should be removed exactly 1 hour after the test meal has been given. A clean rubber œsophageal tube the outside of which has been moistened in hot water is passed gently down the œsophagus into the stomach. The passage is aided by swallowing movements on the part of the patient, who may be lying in bed with his head propped up on pillows or sitting up. Attached to the tube by a glass junction should be a second piece of tubing terminating if desired in a glass filter funnel and the whole must be sufficiently long to allow the funnel to be held well below the level of the stomach. As soon as the tube is in the stomach that is to say has passed freely at least 18 inches beyond the teeth and the resistance of the stomach wall is felt depress the funnel over a clean specimen glass or other receptacle and tell the patient to strain. The contents usually flow out quite readily. If the flow does not commence alter slightly the position of the œsophageal tube by withdrawing it a little or pushing it further in. The flow can sometimes be aided by flattening out a few inches of the tube with the fingers of the two hands and releasing first the portion nearest the stomach thus creating a partial vacuum to suck the contents out. It is never necessary to use a pump. The straining movements of the patient aided if necessary by cautious pressure of the hand on the epigastrium are sufficient to expel the contents. It is wise to have a clean wide receiver ready in case the stomach contents are vomited during the passage of the tube, but this can usually be avoided if the patient is told to refrain from straining until the tube is in its place. After the contents have been withdrawn the stomach can be washed out if such treatment is indicated.

Precautions All medicines must be countermanded for several hours previous to the giving of the test meal. No water should be added to the test meal, and none passed down the tube to start the flow of gastric contents.

(4) *The examination of the gastric contents* Note the amount obtained and the appearance. The microscopic examination of slides made from the contents is of little value.

The estimations of free hydrochloric acid and total acidity are those which give the most important results. They may be considered in three stages.

(1) *Qualitative Test for Free Hydrochloric Acid* (Gunzberg's test)

Required Phloroglucin (or resorcin) Phloroglucin is usually employed, but resorcin works as well and is cheaper.

Vanillin

Absolute alcohol

Small porcelain dish (about 2 inches diameter)

Principle On evaporating a solution of phloroglucin (or resorcin) and vanillin in absolute alcohol with a small quantity of a mineral acid a red colour is formed. As hydrochloric acid is the only mineral acid likely to be present free in the stomach contents any acid detected is almost certainly hydrochloric.

Procedure Filter the gastric contents through a paper moistened with water. To a knife point of phloroglucin (or resorcin) and a knife point of vanillin in a porcelain dish add 1 c.c. of absolute alcohol and 1 drop of the filtrate. Hold in the fingers and heat over a very small naked flame, this is better than heating on a sand bath, as there is less risk of charring.

A rose pink colour in the dish is evidence of the presence of free hydrochloric acid, if this colour is not obtained, free hydrochloric acid is not present.

A reddish brown colour after complete evaporation results from charring and must not be confounded with the rose pink of a positive test.

(2) *Estimation of Free Hydrochloric Acid*

Required Töpfer's solution a 0.5 per cent solution of pure dimethylanilinoazobenzene in alcohol

$\frac{N}{10}$ sodium hydrate

200 c.c. conical flask

10 c.c. pipette

Principle. The filtrate from the gastric contents may contain a mixture of acid compounds, hydrochloric acid, hydrochloric acid in a reversible combination with protein, organic acids and acid phosphates. The filtrate is titrated, using, as indicator, Töpfer's solution, which changes in colour from red to yellow at about pH 3, that is, well on the acid side of neutrality. Since hydrochloric acid is fully dissociated in solution into hydrogen ion and chloridion the hydrogen ion of a solution remains above the change of colour level (and the pH below) and the colour therefore remains red until practically all the hydrochloric acid present is neutralised. The relatively weak organic acids which may be present are only partly dissociated, when their titration is begun, salts are formed which dissociate completely the concentration of the anion of the acid is increased. The dissociation of the acids is therefore repressed and the hydrogen ion concentration falls below that sufficient to give a red colour with the indicator, only small fractions of such acids therefore, are estimated.

The compound of hydrochloric acid and protein breaks up to a certain extent and the hydrochloric acid set free is estimated as free hydrochloric acid.

Acid phosphates do not give a sufficiently high hydrogen ion concentration to affect this estimation.

Procedure. Measure 10 c c of the filtrate into the conical flask, add 30 c c of distilled water and 2 drops of Topfer's solution. If the fluid turns red, titrate with $\frac{N}{10}$ sodium hydrate until the colour turns canary yellow without a trace of orange. The end point is indefinite especially when the amount of organic acids is increased, but it is surprising how closely the results of different workers agree.

If x c c of sodium hydrate are required 100 c c of the test meal contain $10x \times 0.00365$ grams of hydrochloric acid.

The accuracy of this estimation depends on the amount of free hydrochloric acid present the estimates are always too high owing to the protein hydrochloric acid compound. When the concentration of hydrochloric acid is high this error is relatively small, the amount of organic acid present is small, so that the error from this source is relatively very small. When, however, the amount of hydrochloric acid present is low, the amount of organic acid present may be greater, and the relative error from this source becomes large. At the limit,

no free hydrochloric acid may be present, as shown by Gunzberg's reagent, and yet a red colour be given with Töpfer's reagent which may require about 1 c c of sodium hydrate before changing to yellow

An accurate estimation of the free hydrochloric acid present, when this is small, would require an estimation of the hydrogen ion concentration, of the weak acids present, and a complex calculation, it is, therefore, out of the question for practical purposes

(3) *Total Acidity*

Required As for (2), and

Phenolphthalein solution 0.5 per cent in 50 per cent alcohol

Principle All the acids are estimated by carrying the titration to the alkaline side of neutrality

Procedure To the contents of the flask in (2) add 5 drops of phenolphthalein solution, and continue the titration until a definite permanent pink tinge is produced

If y c c of sodium hydrate are used for the titration of all the acids in 10 c c of the filtrate that is that used for (1) plus that used for (2) $10y$ will be required for 100 c c, $10y$ is called the total acidity of the filtrate

(4) *Further Investigations of the Test Meal*

Microscopic investigation has been already referred to. It is remarkable how rarely anything of practical importance is made out by this means

Lactic acid, if present in sufficient amount to give the ordinary tests is abnormal. The presence of lactic acid is practically always associated with a negative Gunzberg reaction and a total acidity of moderate amount, that is from 30 to 40. The demonstration of lactic acid in a test meal is no real additional evidence of carcinoma. Lactic acid is commonly present in carcinoma cases, as well as in chronic gastritis with diminution or absence of HCl

Total chlorides may be estimated in gastric contents by the method used for plasma (p. 78). The significance and diagnostic value of variations of the chloride content are disputed

Pepsin The pepsin content of a test meal is of considerable importance and is readily estimated. Pepsin usually varies

roughly with the amount of the free HCl and the total acidity

The amount of pepsin can be measured by the following method

Required (1) Edestin solution Dissolve 0.1 gram of edestin in a mixture of 30 c.c. of N/10 HCl and 70 c.c. of distilled water warming if necessary Keep in the ice chest (2) Saturated solution of sodium chloride

Procedure In a large test tube place 1 c.c. of filtrate from the gastric contents 25 c.c. of edestin solution and 3 c.c. of distilled water Incubate at 37° C. At intervals of 5 minutes withdraw a small sample and run into 1 c.c. of salt solution When the edestin has been digested completely to edeston an opalescence no longer appears With a normal pepsin content this usually occurs in from 10 to 20 minutes

Blood should not be present in a test meal but may be found in considerable amount in cases of carcinomatous and less often of simple gastric ulcer Blood in quantity visible to the naked eye may be confirmed by microscopic examination blood in less amount should be tested for by the method described under *Fæces* (p. 380)

Bile may be present in the test meal in cases of jaundice and after the performance of a gastro jejunostomy The bile pigments should be tested for in the usual way

Fractional Test Meal

For this test an Einhorn duodenal tube or Ryle's modification thereof is passed until the second mark reaches the patient's teeth A sample of about 15 c.c. of the stomach contents is then withdrawn with a syringe Without withdrawing the tube a test meal is then given consisting of thin gruel made by adding a quart of water to 2 tablespoonfuls of fine oatmeal boiling down slowly to 1 pint and straining through muslin Samples are withdrawn every quarter of an hour for 3 hours After withdrawing each specimen air is injected to empty the tube

The samples are examined for free hydrochloric acid and total acid as described above and tested for starch by the addition of a few drops of iodine solution to each tube

Normally a little hydrochloric acid—not over 0.07 per cent—is present in the resting juice This is partly neutralised by the proteins of the meal so that the concentration of hydrochloric acid is lower in the next specimen a maximum

concentration is reached in about $1\frac{1}{2}$ hours. The reaction for starch disappears in about 2 hours owing to the emptying of the stomach.

The chief advantage of this method is that the time of emptying of the stomach can thus be demonstrated and a delayed secretion of hydrochloric acid which might be missed when only one sample is taken may be detected. Also it is more easy to detect the presence of mucus, bile, blood and remains of previous meals. This however is due to the meal employed and not to the taking of repeated samples.

Histamine Test

The injection of histamine which has a stimulating action on gastric secretion may be used as a means of distinguishing complete achlorhydria from a temporary failure. A stomach tube is passed as for a fractional test meal, the contents of the stomach withdrawn and two further samples taken at intervals of 10 minutes. A dose of 0.75 mgm. of histamine acid phosphate (= 0.25 mgm. histamine) in 1 c.c. of sterile distilled water is then injected subcutaneously. Samples of stomach contents are then withdrawn every $\frac{1}{2}$ hour and examined. The maximum increase in HCl excretion usually occurs after 45 minutes.

Variations in acidity. Free HCl varies in normal individuals between 0.06 and 0.10 per cent. and the total acidity between 40 and 60.

A distinction must be drawn between the condition of complete achlorhydria in which free hydrochloric acid is always absent and conditions in which free hydrochloric acid may be absent with an Ewald test meal but appears at some time in a fractional test meal or those in which free hydrochloric acid may be secreted only in response to an injection of histamine.

Complete achlorhydria is almost invariably found in cases of carcinoma of the stomach. The free HCl may be absent within a very few weeks or even a few days of the onset of symptoms. It is unfortunately the fact that an inoperable carcinoma may be found in an equally short period. In the rare form of carcinoma which appears in a patient who gives a long and definite history of previous attacks of gastric ulcer free HCl may persist. A positive Gunzberg reaction in a patient with a comparatively short history is very strong evidence against a diagnosis of gastric carcinoma.

Carcinoma elsewhere than in the stomach may in a minority of cases the majority of which are cirrhotic lead to an absence of HCl. The total acidity is practically always low in gastric carcinoma. The average total acidity is 26.

In pernicious anæmia complete achlorhydria is almost invariably present. The amount of gastric secretion is scanty and pepsin is present in very small amounts. In the microcytic anæmias of women achlorhydria frequently occurs and hypochlorhydria is the rule. Achlorhydria may also occur in prolonged secondary anæmias.

Achlorhydria without symptoms is not infrequent and often runs in families. The passage of the food through the stomach is rapid and an Ewald test meal may have to be withdrawn in half an hour or less. The secretion is very scanty, the total acidity in the neighbourhood of 10 and pepsin is usually absent.

Free HCl is almost invariably absent after a successful gastro jejunostomy has been performed apparently because of the neutralisation of the gastric juice by admixture with pancreatic secretion. This neutralisation is a most important effect of the operation for the cure of duodenal ulcer. In cases in which free HCl persists after operation peptic jejunal ulcers or a return of the duodenal ulcer may follow.

In cholelithiasis free HCl is frequently absent an important diagnostic point between gall stones and duodenal ulcer. In chronic gastritis free HCl may be absent in the Ewald test meal but the achlorhydria is usually not complete.

Free HCl may be increased in the following conditions —

Pyloric and duodenal ulcers are almost invariably accompanied by a great increase in the free acid and in the total acidity. The average free acidity in a series of cases was 0.17 and the average total acidity was 69. Exceptionally the total acidity may rise above 100. So long as the gastric contents are kept neutralised by drug treatment in these cases the symptoms disappear and complications are most unlikely to occur. Simple gastric ulcer is accompanied by a similar but less marked increase in the acidity. The average of the free HCl is 0.13 and of the total acidity 58. Higher readings are of course frequent. The acidity commonly falls very low and the free HCl may disappear after a considerable hæmatemesis. The fall in acidity is accompanied by an improvement in symptoms.

Hyperchlorhydria or increase in the acidity occurs in the

absence of ulceration and may be very pronounced. It may give rise to symptoms which are readily alleviated by the administration of alkalis but may also be found in apparently healthy persons.

Increase in the acidity is not uncommon in hysterical subjects and may be present in some forms of acute gastritis particularly of the alcoholic type.

Examination of the vomit. The points to be investigated in vomited material—without considering the detection of poisons—are the following—

Blood should be examined for and unless in very obvious amount tested for by the method given under *Fæces*. Among the more important conditions associated with hæmatemesis are cirrhosis of the liver, gastric ulcer simple and malignant and rarely duodenal ulcer. Small quantities of blood in the vomit are of little importance and may be produced by the act of vomiting from a tiny ruptured vessel. Larger amounts may come from elsewhere as from the nose or lungs or mouth and appear after being swallowed. Profuse hæmorrhage from the gastric mucous membrane may follow minute erosions in the absence of macroscopic ulceration and is usually accompanied by hyperacidity.

Bile is of little importance and is likely to occur with any form of vomiting if prolonged.

Fæcal vomiting is characteristic of intestinal obstruction. The vomit is of a brownish black colour resembling that of altered blood and of a fæcal odour. Fæcal vomiting is very occasionally met with in purely functional cases and it may happen with such patients that the odour and colour of a turpentine or soap enema may appear in the vomit.

Mucus in large tough masses may be seen in the vomit in cases of chronic catarrhal gastritis with hypoacidity. It is found in the morning vomit of chronic alcoholic patients.

The acidity of the vomit is in the majority of cases scarcely worth estimating but the expulsion of a quantity of acid fluid some hours after a meal is evidence of hypersecretion.

The bacteriology of the stomach and duodenum. The bacteriological examination of the gastric contents has little clinical bearing upon disorders of the stomach. In films made from the contents bacteria—both cocci and bacilli—are numerous but do not grow upon the ordinary media unless the gastric acidity is too low to give a red colour with Töpfer's

reagent In cases of achylia the common staphylococci streptococci sarcinæ etc, can be readily cultivated The presence of the Oppler Boas bacillus is no longer considered diagnostic of carcinoma of the stomach The bacterial content may be investigated by passing a sterile tube and washing out the stomach with sterile water A sample of the last washing may be taken for examination

The duodenal contents may be obtained after the passage of a small tube in the manner advised for the fractional test meal After passage of the tube the patient is placed on the right side with a pillow under the lower ribs When the bulbous end of the tube has passed through the pylorus the duodenal contents will be recognised by the bile staining Bacteriological examination of the contents often yields bacteria in both films and cultures and the organisms to be particularly examined for are hemolytic streptococci which may be present in the normal duodenum Vaccines prepared from bacteria isolated in this manner are commonly used but are of dubious value

MILK

Analyses of human milk are sometimes required Their value is doubtful as the composition of different samples varies greatly The average composition is fat 2.7 per cent protein 1.2 per cent sugar 6.5 per cent

Fat

Required 100 c.c. graduated stoppered cylinder

Strong ammonia solution alcohol ether and petroleum ether

Pipette made out of glass tubing drawn out at one end The drawn out end is turned up for about $\frac{1}{4}$ inch

Principle All the fat cannot be extracted directly from milk by shaking with fat solvents The fat is therefore converted into an extractable form by the addition of ammonia and alcohol and extracted with ether and petrol ether The fat extracted is weighed

Method Pipette 10 c.c. of milk into the graduated cylinder add 2 c.c. of ammonia solution and 10 c.c. of alcohol Stopper and mix well by inversion Add 25 c.c. of ether and 25 c.c. of petrol ether Mix well by gentle inversion Stand for 1 hour Read the volume occupied by the supernatant layer Pipette

off as large a volume as possible of the supernatant fluid into the Erlenmeyer flask using the special pipette and a large rubber test. Evaporate cautiously on a water bath or in a stream of hot water, dry for a short time at 100°C in an oven cool and weigh.

Calculation If the volume of the supernatant layer was $x\text{ c c}$, if $y\text{ c c}$ were pipetted off, and the increase of weight of the flask was z grams, the milk contained $\frac{10xz}{y}$ grams of fat per 100 c c

Protein

Required 10 c c pipette, 2 c c pipette

2 c c microburette

8 × 1 in pyrex test tubes

Saturated solution of potassium oxalate

0.5 per cent alcoholic solution of phenolphthalein

1 per cent alcoholic solution of acid fuchsin

N/10 NaOH

Commercial formalin

Principle Neutral formaldehyde is added to the milk. It combines with the basic $-\text{NH}_2$ groups forming a neutral compound and thereby causing an increase of acidity proportional to the amount of protein present.

Method To 10 c c of milk in a test tube add 0.4 c c of saturated potassium oxalate solution and 0.5 c c of phenolphthalein solution. Shake and stand for 2 minutes. Add N/10 NaOH until the colour is the same as that of an equal quantity of milk to which 1 drop of alcoholic fuchsin solution has been added (this sample may be used for the fat estimation). Add a drop of phenolphthalein solution to about 3 c c of formalin and add N/10 NaOH to this carefully until a pink colour appears. Add 2 c c of this neutral formalin to the neutral milk. The pink colour disappears. Titrate with N/10 NaOH until the colour again matches the milk to which fuchsin has been added.

Calculation If $x\text{ c c}$ of N/10 NaOH are required the milk contains $1.74x$ grams of protein per 100 c c

Sugar

Required As for blood sugar

Principle As for blood sugar, but owing to the higher concentration of sugar in milk the filtrate must be diluted, and

owing to the different reducing power of lactose the calculation is different

Method Into a small flask run 2 c c of milk, 16 c c of distilled water, and 1 c c of sodium tungstate solution Mix and add 1 c c of $\frac{2}{3}$ NH_4SO_4 with shaking Shake well, stand and filter, the filtrate should be clear Dilute the filtrate 25 times with distilled water and proceed as in blood sugar estimation

Calculation If the standard is set at 20 mm and the reading of the unknown is x the milk contains $2.5 \times \frac{20}{x} \times 1.4$ grams of sugar in 100 c c, if standard I was used, twice this if standard II was used

CHAPTER XX

THE PANCREAS—THE LIVER—THE SPLEEN— THE PERITONEUM

THE PANCREAS

Pancreas Tests of the pancreatic efficiency other than those concerned with the function of the islets designed to detect pancreatitis or obstruction to the pancreatic duct are all unsatisfactory. With deficiency of pancreatic secretion or failure of the secretion to reach the intestine the digestion of protein and fat is impaired. Failure to digest protein may be detected by an increase of nitrogen in the faeces with the patient on a diet of known composition but it is usual to trust to the finding of striated muscle fibres in the faeces—when the patient's diet contains meat. This method will detect complete absence of pancreatic juice but its value in diagnosis of chronic pancreatitis is doubtful.

Impairment of the digestion of fat leads to an increase of neutral fat in the faeces (p. 380). However with variations of the amount of fat in the diet great variations from the normal standards as usually given may occur without any disease of the pancreas and unless the diet is standardised no conclusions can be drawn except from gross abnormalities. These are usually found only when obstruction is present and such changes can as a rule be detected by mere inspection of the dried faeces.

In acute pancreatitis and sometimes in chronic pancreatitis the diastatic index of the plasma and urine (p. 329) is greatly increased. Such an increase may be taken as certain evidence of a pancreatic lesion but a normal diastatic index cannot be regarded as evidence that the pancreas is sound.

Various tests based on the necessity of pancreatic juice for the digestion or splitting of various substances such as salol have proved untrustworthy.

Pancreatic cysts and the methods of testing for enzymes in them have been mentioned in the section on cysts (p. 280).

Damage to the *islets of Langerhans* which may or may not be associated with disease of the rest of the pancreas shows itself by impairment of the power of metabolising carbohydrates. This impairment may take the form of frank diabetes mellitus or be detectable only by the *glucose tolerance test*, this test should therefore be used when disease of the pancreas is suspected.

It is performed as follows. In the morning the patient who has taken nothing but water since the previous evening is bled for sugar estimation then drinks 50 grams of glucose dissolved in 100 c.c. of water and flavoured with tartaric acid. He is bled again after $\frac{3}{4}$ hour and after 2 hours. With children the

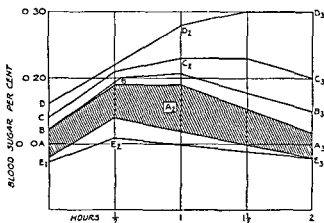


FIG. 26.—Glucose Tolerance Test

dose of glucose should be reduced to 0.5 gram per pound of body weight.

The blood sugar normally rises as shown (A₁, A₂, A₃) in Fig. 26. The height of the peak is variable but the blood sugar almost always comes down to or below the fasting level in 2 hours. As is usual with efficiency tests the results obtained with any patient are rarely quite so uniform as those with perfectly normal persons and the variations as shown here are greater than can be considered strictly normal.

With various degrees of reduction of the power to metabolise glucose (lowered carbohydrate tolerance) curves such as B₁, B₂, B₃, C₁, C₂, C₃, D₁, D₂, D₃ are obtained. The maximum is higher and reached later than in the normal curves and the return to the fasting level is delayed beyond 2 hours.

Such curves are obtained not only in cases of diabetes mellitus but also in diseases of the glands of internal secretion hyperpituitarism exophthalmic goitre (C_1 C_2 C_3) myxoedema (B_1 B_2 B_3) frequently also in old age (B_1 B_2 B_3) hence the common occurrence of one form of alimentary glucosuria. This latter term is one that causes confusion as it is used for a glycosuria due to excessive intake of carbohydrate diminished power of metabolising carbohydrate or low renal threshold.

With renal glycosuria a normal or low curve such as E_1 E_2 E_3 may be obtained.

The urine may be collected in $\frac{1}{2}$ hour samples during the test and examined for glucose.

Increased sugar tolerance shown by a low curve (E_1 E_2 E_3) is sometimes found in hypopituitarism (Frohlich's syndrome).

Apart from the diagnosis of frank cases of glycosuria this test is of value in clearing up doubtful cases with some real or alleged reducing substance in the urine. If the patient has a low threshold or a slightly reduced tolerance leading to slight or intermittent glycosuria the condition will be made manifest by this test and two of the possible conditions can therefore be diagnosed or excluded.

Liver function tests. It might be expected that several of the functions of the liver would lend themselves to efficiency tests but actually only two tests appear to give useful information these are the levulose tolerance test and the excretion of phenoltetrachlorophthalein. The latter is so dangerous owing to the frequent occurrence of thrombosis following intravenous injection that it cannot be recommended. The less toxic dye bromsulphonaphthalein appears to give less indication of the state of the liver.

Levulose tolerance test. This is done in the same way as the glucose tolerance test but instead of glucose 40 grams of levulose are given. In normal persons the rise of blood sugar is slight (A_1 A_2 A_3 Fig 27) and an increase of 0.03 per cent or more is evidence of liver damage (B_1 B_2 B_3) with severe damage much greater increase may be found (C_1 C_2 C_3).

It is essential that the levulose used should be pure. It must be remembered that in conditions in which the glucose tolerance is reduced abnormal curves will be obtained with levulose also whether the liver is damaged or not.

Urobilin. When excessive formation of bilirubin such as occurs with increased breakdown of red blood corpuscles can

be excluded the presence of urobilin in abnormal amounts in the urine may be regarded as evidence of liver damage as can also excess of bilirubin in the plasma, when excessive formation of this pigment and obstruction of the bile passages can be excluded

Tyrosine in urine In acute yellow atrophy of the liver and in phosphorus poisoning excess of leucine and tyrosine may be found in the urine

The presence of excess of tyrosin in the urine when no crystals are found can be detected as follows. Take 200 c c of urine and remove coagulable proteins if present by boiling and filtering. Add basic lead acetate solution 5 c c at a time with shaking until no further precipitate forms—this removes

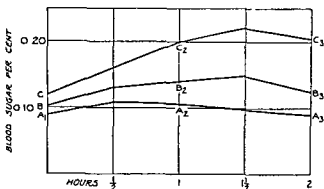


FIG. 27—Lævulose Tolerance Test

the urinary pigments and other substances. Let stand overnight, filter. Pass H_2S through the filtrate to precipitate lead and filter off the precipitate. Evaporate the filtrate to a small bulk on a water bath. If over 0.5 gram of tyrosin is present per litre, crystals will separate out on cooling. These can be identified by heating with water and adding a few drops of Millon's reagent to the hot solution, which becomes rose-coloured in the presence of tyrosin.

In cases of **biliary obstruction** the patient is, as a rule, obviously jaundiced and the condition may be confirmed by the demonstration of bile pigment in the serum. The pigments may also be present in the urine, and if obstruction is complete, absent from the stools. The serum in cases of biliary obstruction is often markedly bile tinged at a period when clinical evidence of jaundice is lacking.

Puncture of the liver is occasionally performed in cases of suspected liver abscess or of hydatid cyst. Except during the course of an operation, puncture of the liver should be performed cautiously and only in the area of an evidently superficial tumour. A fine hypodermic needle should be used. The fluid withdrawn from a tropical abscess is very typical in appearance, having a considerable resemblance to anchovy sauce. Unless a secondary infection has occurred the fluid is odourless. Amœbæ should be looked for in fresh preparations of the fluid, but are rarely detected, since they seem to be mainly confined to the lining wall of the abscess. After the abscess has been opened and drained, the amœbæ are likely to appear on the second or third day. The methods of detecting amœbæ in the stools should be applied in the examination of 'pus' from the liver. Dried films made from the pus show little besides necrotic cells and amorphous debris. Cultures are almost invariably sterile.

The nature of the fluid of a hydatid cyst has been described in the section on puncture fluids. The characteristic hydatid hooklets should always be sought for.

Bile is occasionally obtained by puncture, through the abdominal wall, of a distended gall bladder; it is more frequently and justifiably received in the laboratory from the operating theatre. In cases of distension of the gall bladder due to a calculus impacted in the cystic duct, the fluid is often colourless or merely tinged with bile pigment, proteins are scanty and mucin is usually present in considerable amount. If no distension of the gall bladder is present the normal bile is greenish in colour and of a glycerine like consistence. When inflammation is present or gall stones are found in addition, the bile may be either paler or darker in colour, and is almost always more syrupy andropy in consistence. More rarely the gall bladder may be filled with pus, and actual gangrene of the walls may be present. The bile in such cases should be examined by cytological and cultural methods. Gall stones if present should be culturally examined and their composition investigated.

In about 20 per cent of cases with gall stones the bladder is found to be sterile, and formerly considerable importance was attached to the antiseptic properties of the bile in preventing the spread of organisms from the alimentary tract.

It is probable that the antiseptic action of the bile salts is

of importance, but it has little effect upon organisms of the colon and typhoid groups. The antiseptic action of the bile is diminished under various pathological conditions, and organisms other than those of the coli typhoid group may be found.

The following are among the organisms commonly met with in the gall bladder —

The *bacillus coli* is the organism most frequently present in inflammatory conditions of the gall bladder either alone or in association with gall stones. There is no difficulty in recovering and identifying it in cultures made from the bile by the ordinary methods.

The *typhoid bacillus* may be present in the gall bladder many years after an attack of typhoid fever. The association of typhoid fever with the subsequent production of gall stones is well known. The detection of typhoid bacilli in the gall bladder is of great importance apart from the production of calculi since the latency of the organisms in this situation is probably responsible for the majority of instances of typhoid carriers. The organisms readily pass down the intestinal tract and are excreted in the fæces. Such a typhoid carrier occupying the position of a cook or a domestic servant is a serious menace to the community with which he or she is associated. The detection of typhoid bacilli in the bile is a simple matter, if the bile is available during an operation for gall stones. Typhoid carriers can otherwise be recognised by the detection of the bacilli in the stools and by examination of the serum for the presence of typhoid agglutinins.

Streptococci, *staphylococci* and much more rarely *pneumococci* are among other organisms which may be obtained in cultures from the bile.

Gall stones may be either single or multiple. If multiple they are usually faceted. They may be found in the gall bladder, cystic duct or common duct or they may have escaped into the intestine. Rarely they may have ulcerated through into the peritoneal cavity or through the anterior abdominal wall. Exceptionally small calculi may be found in the finer hepatic ducts in the substance of the liver. The great majority of gall stones consist mainly or entirely of cholesterol. Pure cholesterol calculi are of a translucent yellow colour and very light. The stones not infrequently contain in addition to the cholesterol varying amounts of biliary pigment either

bilirubin or biliverdin. More rarely small calculi are found consisting entirely of biliverdin or bilirubin with a small admixture of calcium. Calcium carbonate and phosphate calculi are common in some animals, but very rare in man.

Analysis of Gall stones

Powder the stone. Shake some of the powder with ether. Pipette off the ether and evaporate in a dish on a water bath. Test the residue for *cholesterol* as follows —

(a) Redissolve some of the residue in ether. Allow a few drops to evaporate on a slide, and examine for needle shaped crystals of cholesterol.

(b) Dissolve another portion in alcohol and evaporate on a watch glass. Examine for cholesterol crystals—rhombic plates notched at the edges.

(c) Dissolve another portion in about 2 c c of chloroform in a dry test tube, add 0.5 c c of acetic anhydride and 0.2 c c of pure strong sulphuric acid. A bluish green colour shows the presence of cholesterol.

If any residue is left after ether extraction add to it dilute hydrochloric acid. If carbonates are present effervescence occurs. Heat, filter off any insoluble residue and make the filtrate alkaline with strong ammonium hydroxide. A precipitate will form if *phosphates* are present. Acidify with glacial acetic acid, phosphates if present will redissolve. Add ammonium oxalate solution, a precipitate shows the presence of *calcium*.

Dissolve another portion of the residue in strong nitric acid, add excess of 10 per cent ammonium molybdate solution and boil. A yellow precipitate shows the presence of phosphates.

The presence of a small amount of bile pigment in cholesterol stones is shown by a yellowish brown colour. The large amounts in biliverdin calcium stones give them a greenish black colour.

The bacteriological investigation of gall stones is conducted as follows —

If the stone has been removed by the surgeon from the bile passages and placed direct in a sterile receptacle no preliminary treatment is necessary. If, however, the surface of the stone has become contaminated it should be placed on a clean dish and washed repeatedly with sterile water. The stone is then placed in a sterile mortar and crushed. Culture tubes are

inoculated with portions of the crushed calculus. The remainder can be used for chemical analysis.

The bacteria found in gall stones are precisely similar to those present in the bile. A considerable percentage of gall stones, however, are sterile.

THE SPLEEN

Tumours in the left hypochondrium continue to furnish the clinician with interesting problems for diagnosis. If such a tumour be present several pathological investigations may be necessary.

It is not intended to imply that all these investigations should be performed in every case of splenic enlargement. The clinical examination of the patient will almost always indicate which investigation is necessary.

Examination of the cells of the blood. In all cases of splenic enlargement some examination of the blood must be made, and this in a high percentage of cases will establish the diagnosis.

In myeloid leukaemia, among the primary blood diseases, there is almost always a considerable enlargement of the spleen, and the enlargement is often so great that the spleen comes to occupy more than half of the abdominal cavity.

In lymphoid leukaemia the spleen is as a rule moderately enlarged, but occasionally the enlargement is insufficient for detection by clinical means.

In pernicious anaemia the spleen is enlarged and commonly of such a size as to be just palpable.

Enlargement of the spleen from any of the above three causes is readily recognised by the ordinary examination of the blood.

In chlorosis there is no evident enlargement of the spleen, and if considerable splenic enlargement is present with an anaemia of the chlorotic type some other cause for the splenomegaly must be sought.

In erythraemia a considerable increase in the size of the spleen is usual. Patients with this condition are, as a rule, cyanosed, and the blood shows great excess in the number of red cells.

Enlargement of the spleen in small children may be associated with active rickets or with congenital syphilis, and

with little change in the blood count, or these diseases may be absent and the blood show the pronounced, if variable, changes of the splenic anæmia of children

Occasionally marked enlargement of the spleen in children may occur in the absence of other obvious disease or of changes in the blood

In primary splenomegaly or splenic anæmia of adults, and in Banti's disease, the size of the spleen is usually very considerable, and may even rival that found in myeloid leukaemia. The changes in the blood in this condition are not diagnostic, but if a considerable anæmia of the secondary type is absent and there is no leucopenia, the diagnosis of primary splenomegaly is probably incorrect

In hæmolytic icterus the size of the spleen is considerable. The condition may be recognised by the abnormal fragility of the red cells in dilute saline

In acute inflammatory conditions a slight enlargement of the spleen is frequently met with. The blood shows an increase in the total leucocytes, with a relative increase in the polymorphonuclear neutrophils and large hyalines

Examination of the blood serum The moderate enlargement of the spleen which occurs in typhoid fever, and the more marked enlargement present in Malta fever, may be confirmed by the demonstration of the specific agglutinins in the serum

Enlargement of the spleen is often considerable in congenital syphilis and in the amyloid degeneration which may follow congenital or acquired syphilis. The spleen may be palpable in the secondary stage of syphilis. A positive Wassermann reaction will be found in the serum

The parasitology of the blood Definite enlargement of the spleen may follow infarction in the course of infective endocarditis, and the nature of the disease may be established by cultivation of the blood. In relapsing fever the spirillum is present in blood films. In malaria a moderate enlargement of the spleen is usual, and the parasites can nearly always be found in film preparations. The enlargement of the spleen which accompanies malaria may exceptionally persist for a considerable time after the patient has left the malarial district. In such cases no parasites may be found in the blood. A very considerable enlargement of the spleen without the typical signs of malaria and in the absence of malarial parasites in the blood is probably due to some other cause. A past history of

malaria is not necessarily the explanation of a tumour in the left hypochondrium

Spleen puncture should be performed in cases of splenic enlargement which may possibly be the result of kala azar. The puncture is without risk if performed with a fine hypodermic needle. Sufficient material can usually be withdrawn to demonstrate the Leishman Donovan bodies.

The urine must always be carefully examined in all cases of tumour in the left hypochondrium. A renal swelling on clinical examination may very closely resemble an enlarged spleen and the detection of pus in the urine may serve to clinch the diagnosis. If the spleen is enlarged from amyloid disease the kidneys will probably also be affected and the urine will contain albumin often in considerable amount.

Cirrhosis of the liver is often associated with a hard and palpable spleen. The diagnosis as a rule is readily made on clinical grounds.

Hodgkin's disease is frequently associated with a splenic enlargement which may be considerable. None of the examinations just described are likely to throw any light upon the diagnosis. A general enlargement of the glands however is nearly always present and it is justifiable to remove a single superficial gland for histological investigation.

Tuberculosis particularly of the generalised glandular type may lead to considerable splenic enlargement. Laboratory investigations in the absence of a satisfactory complement deviation test for tuberculosis are mainly negative. The diagnosis is often arrived at by a process of exclusion and commonly rests between tuberculosis and Hodgkin's disease.

Tumours of the spleen whether primary or secondary are extremely rare. Cysts of the spleen and gummata of the spleen are likewise in the nature of pathological curiosities.

Renal tumours, tumours of the stomach or intestine and tumours or cysts of the omentum, mesentery, pancreas or ovary may all closely resemble splenic tumours on physical examination.

It is evident that a large number of diseases may be accompanied by splenic enlargement. In the majority of them however the pathological condition is fairly obvious on clinical grounds and the enlargement of the spleen is of secondary importance. Particularly is this the case in slight enlargement of the spleen to palpation. Great enlargement of the

spleen is rare and the most usual causes are myeloid leukaemia splenic anaemia kala azar the splenic anaemia of children and chronic lymphoid leukaemia These diseases can nearly always be recognised by a blood examination or by spleen puncture Moderate enlargements of the spleen are more difficult of diagnosis and the commonest causes are tuberculosis Hodgkin's disease and in children congenital syphilis and rickets

THE PERITONEUM

The cellular nature of peritoneal exudates has been sufficiently indicated in the section dealing with puncture fluids The bacteriological examination of exudates obtained at operation may be further considered in reference to the abdominal lesion responsible for the inflamed peritoneum

Gastric ulcer Perforation of a gastric ulcer may lead to a localised abscess or to a generalised peritonitis A number of different organisms may be found in the exudate but in the majority of cases the bacterium which predominates in film preparations and often in cultures is a streptococcus usually of the viridans type The predominance of a streptococcus in cases of perforated ulcer helps to distinguish them from lesions lower in the gut where the exudate is almost entirely bacillary A similar streptococcus is often present in the pus of a perinephric abscess It must not be supposed that the presence of a streptococcus in the peritoneum in a case of perforated gastric ulcer indicates that the ulcer was due to streptococcal inflammation Streptococci of a similar nature may be found frequently in the gastric contents in the absence of ulceration and whatever may be the cause of gastric ulcer the effect of perforation is to give to the organisms which may have been in the stomach access to the peritoneum The same warning must be applied to all bacteriological findings in peritonitis following visceral lesions Among other organisms which may be found in this condition are staphylococci and *B coli*

Duodenal ulcer The bacteriology of the peritoneum after perforation of a duodenal ulcer is similar to the foregoing but streptococci are less commonly and staphylococci and colon bacilli more commonly met with

The ileum The most important variety of perforation of the ileum is that which follows a typhoid ulcer the most

common site of perforation being within the last foot of the ileum. Failure to find the typhoid bacillus in these cases is common, and is no evidence against the specific cause of the intestinal ulceration. Colon bacilli, staphylococci and streptococci are recovered more frequently than the typhoid bacillus, and possibly in the majority of cases take a greater share in the peritoneal inflammation. Similar organisms (with the exception of *B. typhosus*) are found in the peritoneum after perforation of the ileum from injury or other causes.

The appendix. In cultures made from the lumen of an inflamed appendix, from a localised appendix abscess, from the general peritonitis accompanying a diseased or perforated appendix, and from the residual and remote abscesses which may follow appendicitis the predominant organism found is in the great majority of cases the colon bacillus. It is open to doubt, however, whether anything approaching this proportion of appendicular inflammation is produced by the same organisms which cause the peritonitis. There is no evidence of any specific cause of appendicitis, and it is possible that a variety of organisms may be responsible and that as soon as a communication has been established between the lumen of the gut and the peritoneal cavity the colon bacillus rapidly multiplies and takes the predominant share in the subsequent peritonitis. There is abundant evidence that the colon bacillus in pure culture is able to set up a virulent peritonitis. In addition to the colon bacillus other organisms are frequently present in film preparations. Long, thin and sometimes beaded bacilli are often found and are probably the cause of the offensive smell so often met with in the pus of an appendix abscess. These organisms do not grow in aerobic cultures, and not always under anaerobic conditions. They are probably identical with the organisms found in stinking empyemata and in some cerebral abscesses, and their rôle appears to be mainly that of saprophytes. Their normal habitat is the alimentary or respiratory tract. Staphylococci and streptococci are commonly met with in film preparations in association with the colon bacillus, particularly in localised appendix abscesses. They may also be isolated in culture and are occasionally present in pure culture. The *bacillus pyocyaneus* is less frequently met with, but may be obtained in pure culture.

Lesions of the large intestine may originate a peritonitis, as after perforation of a simple or a malignant ulcer. The

organisms found in the peritoneal exudate are of much the same type as those met with in appendix cases

The pelvic organs in the female may cause peritonitis following suppuration in one of them. A pyosalpinx rarely ruptures, but when it does peritonitis usually follows. The pus is almost invariably sterile, but very occasionally the gonococcus can be identified in film preparations after considerable search. Recovery in such cases is the rule.

In the case of suppuration in ovarian cysts or tumours a peritonitis of the ordinary type is set up, and colon bacilli, streptococci, and staphylococci are found in the exudate. Not infrequently the pneumococcus is isolated in pure culture from the pus in the neighbourhood of an ovarian abscess, and the prognosis appears to be favourable.

Post-operative peritonitis is now fortunately of rare occurrence but even in the most careful hands occasional instances of operative infection occur. In the pus of such cases the *streptococcus hæmolyticus* may be isolated in pure culture and a rapidly fatal course is to be expected. Less virulent staphylococci are found in some cases, and in others colon bacilli or *B. pyocyaneus*.

Intestinal obstruction. The commonest variety of intestinal obstruction is that which follows strangulation of a hernia. In cultures made from the general peritoneal cavity staphylococci are frequently obtained, and less commonly colon bacilli. The more damaged the gut, the more frequently are colon bacilli obtained and the more serious is the prognosis. A considerable amount of free fluid is often found in the hernial sac. In the majority of such cases this fluid is quite clear, contains few cells other than endothelials, and cultures from it remain sterile. If the gut in the sac is gangrenous, organisms are more likely to be obtained in culture.

In cases of obstruction due to other causes the probability of obtaining sterile cultures from the peritoneum depends upon the chronicity of the process. The more acute the case, the more commonly are organisms found.

"Idiopathic peritonitis." This term was formerly applied to cases of peritoneal inflammation without any recognisable focus in the intestine. An important organism met with in such cases, and often in pure culture is the pneumococcus. Pneumococcal infection of the peritoneum is more common in children than in adults, and females are more frequently

affected than males. A pneumococcal infection of the lung may be present, but more commonly no such local focus is found, and the organism appears to have entered the peritoneum from the general circulation. In young girls the pneumococci possibly enter by way of the genital tract. The organisms may be present in pure culture in the interior of the uterus, and are among the causative bacteria to be met with in cases of ovarian suppuration.

Streptococcal peritonitis due to causes other than intestinal may develop as a terminal infection in several chronic diseases, of which chronic nephritis is the most common. Peritonitis may also develop as part of a general streptococcal septicæmia with a primary focus elsewhere. More rarely a streptococcal peritonitis may be present as a local disease and without any traceable source of infection.

The prognosis in those cases in which pneumococci are found in the peritoneum is much more favourable than those associated with streptococci. The streptococcal cases are almost invariably fatal.

Tuberculous peritonitis. In tuberculous peritonitis with effusion the fluid is commonly quite clear, and the predominant cell is a small lymphocyte. Mixed cellular fluids are, however, common in the peritoneal cavity, and a percentage of lymphocytes is found in other than tuberculous conditions. Secondary infection of a tuberculous peritonitis is fairly frequent, and the fluid may be turbid or purulent and contain a considerable percentage of polymorphonuclear cells. Staphylococci are often found in cultures taken from such cases.

Tubercle bacilli are usually difficult to find, but may be very numerous, and should be sought for by the ordinary methods. If the bacilli cannot be found the fluid can be proved to be tuberculous by injection into a guinea-pig.

CHAPTER XXI

THE FÆCES

It is remarkable how much the clinical investigation of the fæces has fallen behind the physiological knowledge of the excreta. Notwithstanding the interest attached to numerous intestinal disorders and the importance attributed to the condition known as intestinal toxæmia, the usual investigation of the stools consists of little more than a perfunctory inspection. The main reason of this neglect is that very few simple clinical investigations are known to throw any direct light on the condition of the patient. Elaborate chemical analyses of the excreta require that the patient should be dieted in a manner impossible in the ordinary hospital ward, and the analyses themselves are too prolonged for ordinary routine work. A certain number of minor investigations, however, such as are within the scope of everyday work, are of considerable use in some diseases and essential for diagnosis in others.

Such methods of examination as are given here are divided into naked-eye, chemical, microscopical, and parasitological investigations.

In most investigations it is advisable that the ordinary stool of the patient should be examined when available, and not the stool which follows the administration of a purgative or the giving of an enema.

The Naked-eye Investigation

The amount and the appearance of the fæces naturally vary considerably in health. The quantity passed by a healthy man on an average diet in the 24 hours is said to be about 100 grams.

The colour of the stools is very variable, and the colour in health is due to the presence of the pigment stercobilin. Stercobilin is very similar to the urinary pigment urobilin, and is considered to be identical with it. The two bodies give the same chemical tests. Urobilin, or a very similar sub

stance can be artificially produced from bile pigment and there is little doubt that the stercobilin of the fæces is formed from bilirubin in the alimentary canal. Unaltered bile pigment is never present in the fæces in health. The colour of the fæces may be darkened by an excess of stercobilin or after the taking of medicines containing iron, manganese or bismuth. The blackening of the stools as the result of drugs is due to the formation of the sulphides of the metals in the alimentary tract. Blood coming from the lower part of the bowel may be of the usual red colour but if from the upper part it is black and the stools resemble closely those which follow the taking of iron or bismuth and can only be distinguished by a chemical examination. Green stools may under abnormal circumstances be due to the presence of unaltered bile pigment. Yellow stools follow the administration of senna, santonin and rhubarb. The colour of the fæces is lost if the bile is prevented from entering the intestinal canal and the characteristic clayey stools of jaundice are passed. Light coloured stools due to a diminution of stercobilin are passed in various conditions associated with diarrhoea. They do not necessarily contain an excess of fat but cannot be distinguished by the naked eye from the fatty stools suggestive of pancreatic disease.

The odour of the fæces is due mainly to the presence of indole and scatole. The offensiveness varies with the nature of the food—being much greater with a meat than a carbohydrate diet—and with the amount of intestinal putrefaction.

The consistence of the stool naturally passed is of great importance. The formed stool of health is replaced by the more or less watery evacuation of diarrhoea from a large number of causes. An unformed stool in an adult on an ordinary diet is always abnormal irrespective of the number of the motions in the 24 hours.

Abnormal Ingredients

Numerous abnormal substances in the stools can be detected by the ordinary inspection. In cases where abnormal substances are to be expected the examination is greatly facilitated by repeatedly washing the stool in tap water through a fine sieve and inspecting the residue. The following are among the abnormal substances to be looked for.

The larger animal parasites are easily detected with the naked eye. They have been described previously (p. 153).

Mucous shreds and mucous casts are of considerable importance and are readily seen in the residue of the stool floated in water. They are commonly met with in numerous varieties of colitis and consist as a rule of thin ragged membranes of varying size composed of mucin and fibrin. Large mucous casts of the alimentary tract are less commonly seen and may appear as long twisted strands which bear some resemblance to tape worms. The strands are not regularly segmented and should be readily distinguished from parasites. Portions of undigested food and particularly orange and banana fibre closely resemble mucoid shreds but can be differentiated under the microscope. Banana fibres have some microscopic resemblance to worm segments filled with ova.

Shreds of epithelium and rarely particles of malignant growth may be met with and should be reserved for microscopical examination.

Pus and blood may be present in amount obvious to the naked eye but in nearly all cases the microscopic evidence should be confirmed by other means.

Gall stones are to be carefully looked for in all suspicious cases and are readily extracted from the feces by the sieve process described above. Gall stones have to be distinguished from scybalous particles of feces and after washing in water they must be chemically tested for cholesterol. Cholesterol is normally present in the feces but a solid macroscopic object in the feces consisting almost entirely of this substance is certainly derived from the gall bladder. Faecal concretions are as a rule more friable and pultaceous contain very little or no cholesterol and often a considerable amount of carbonates or phosphates.

Intestinal sand is occasionally passed in considerable amounts in some forms of colitis. True intestinal sand is of a brownish colour and is composed mainly of calcium carbonate and calcium phosphate. Cholesterol is absent. The colour is due to stercochrom often with traces of bile pigment. False intestinal sand consists as a rule of particles of undigested vegetable fibres and most commonly follows the ingestion of pears.

Chemical Examinations

The reaction The reaction of the stool to litmus paper has no particular significance. The normal stool may be either faintly acid or faintly alkaline. Marked acidity or alkalinity is abnormal.

Stercobilin is probably identical with urobilin and can be detected in the fæces as follows —

Extract about 3 c c of fæces or preferably about 0.5 gram of dried fæces in a test tube with ether. Pour off the ether and extract the residue with 5 c c of alcohol containing 2 drops of strong hydrochloric acid. If stercobilin is present the extract will be brown and show an absorption band in the green. Neutralise the extract with ammonia. In another tube shake about 0.5 gram of zinc acetate with 5 c c of alcohol. Mix the contents of the two tubes well by pouring from one to the other. Stercobilin will give a pinkish solution which shows a green fluorescence against a dark background. This zinc acetate test is not so satisfactory as that for urobilin in urine.

Stercobilin is absent in cases of complete occlusion of the bile ducts. It is present if the occlusion is incomplete, however deeply jaundiced the patient may be. The test is thus a satisfactory one for the demonstration of complete occlusion of the bile ducts.

Bile. Bile pigment is normally absent from the fæces. It is present in some cases of diarrhœa and traces are found in conditions associated with jaundice.

Bile pigment may be tested for by Gmelin's test applied to a watery mixture of the fæces or a particle of fæces may be mixed with a concentrated solution of mercuric chloride, covered and allowed to stand for 24 hours. The normal stool coloured by stercobilin turns red. If bile pigment is present green particles appear.

Bile acids may be tested for as follows —

Extract the fæces with alcohol. Dissolve the residue in dilute caustic soda and perform Pettenkofer's test thus —

Dissolve a fragment of cane sugar in the solution of the residue in a test tube.

Run in about 5 c c of concentrated sulphuric acid to the bottom of the tube and gently shake.

A slowly developing purple colour forms from the line of junction of the two fluids.

Excess of cane sugar in the mixture must be avoided since it is charred by the acid and so masks the reaction.

Blood Pigment. Blood present in considerable amount and coming from the lower part of the tract may be detected by the microscope or by its spectrum.

Blood in small amount or from the upper part of the tract

cannot be recognised under the microscope, and is best examined for by one of the tests for so called "occult" blood. By occult blood is meant blood present in minute traces, such as may come from an oozing gastric, duodenal, or malignant ulcer. Various delicate tests capable of revealing very minute traces of blood are employed and are of some assistance in the diagnosis of alimentary conditions associated with hæmorrhage. Before performing such a test it is necessary to put the patient on a blood free diet for 48 hours.

✓ *Benzidine Reaction for Blood Pigment*

Required (1) Benzidine (2) Sodium perborate. It is convenient to use the tablets, which can now be bought, containing 0.1 gram of each. (3) Glacial acetic acid.

Procedure Grind one of the tablets and dissolve in a mixture of 5 c.c. of glacial acetic acid and 5 c.c. of water. Filter. Place 2 drops of the filtrate on a clean slide. To one of the drops add a platinum loop of the material to be tested, if it is fluid, or of the material rubbed up with a little saline, if it is dry. If blood is present a green colour appears in this drop in less than 1 minute, the time of appearance depending on the amount present. The second drop serves as a control.

This test for blood may be applied to other materials—urine, test meals, suspected blood stains.

Fat The neutral fats, that is glycerides of the higher fatty acids in the food are split by the lipase of the pancreatic juice into glycerol and fatty acids. Some of the fat escapes splitting, and some of the fatty acids formed are not absorbed, and partly combine with bases to form soaps, this unsplit fat and unabsorbed fatty acid and soap appear in the fæces. The three forms can be estimated sufficiently accurately for clinical purposes by Cammidge's method.

Required

Two Schmidt-Werner tubes

Two 25 c.c. conical flasks

Ether, alcohol, $\frac{N}{10}$ alcoholic sodium hydrate

Hydrochloric acid, 1 part in 2 parts distilled water

Phenolphthalein solution

Principle The neutral fats and free fatty acids, which are soluble in ether, are extracted from the fæces, which have been dried on a water bath. The ether is evaporated and the

extracted fat and fatty acid weighed. This extract is then dissolved in alcohol titrated with $\frac{N}{10}$ alcoholic sodium hydrate and the weight of free fatty acid reckoned as stearic acid is calculated from the titration figure. The neutral fat is given by difference. The fatty acid of the soaps is split off by heating with hydrochloric acid and extracted together with the neutral fat and free fatty acid as before and weighed. By subtracting the neutral fat and free fatty acid the weight of fatty acid combined as soaps is obtained.

A certain amount of substances resembling cholesterol are extracted with and behave as neutral fat so that the figure obtained for neutral fat is actually too high.

Procedure A sample of the usual stool is taken the patient being given an ordinary mixed diet.

The faeces are placed in a porcelain evaporating dish and heated over the water bath in a fume cupboard. The faeces are stirred occasionally with a glass rod and the heating process is continued until they are thoroughly dry. The process takes some hours as a rule and may be completed by leaving in a drying chamber over night. When cool the dried faeces are practically inoffensive.

The dried faeces are powdered into as fine a dust as possible in a mortar. It may be impossible to powder very fatty stools.

Two samples each of 0.5 gram are carefully weighed out.

Two clean and absolutely dry Schmidt Werner tubes are prepared and labelled A and B. Each should be provided with a 10 c.c. mark.

Into the lower bulb of each place 0.5 gram of faeces.

Wash the residue into the A tube with 1 in 3 hydrochloric acid and fill up with the acid to the 10 c.c. mark.

Wash the residue into the B tube with distilled water and fill with water to the 10 c.c. mark.

In each tube all the faeces must be collected with the fluid in the lower bulb.

The A tube is then heated in boiling water for 15 minutes and is rotated from time to time. By this process the fatty acids are split off from their bases and rendered soluble in ether.

Cool the A tube.

Fill both tubes to the 50 c.c. mark with ether and cork them securely.

Slowly invert and rotate each tube forty times.

Allow the tubes to stand for 30 minutes or until all the residue has collected into the lower bulbs

While the tubes are standing weigh carefully two small dry evaporating flasks labelled A and B. Note the weight of each. Into each appropriate flask measure 20 c.c. of the clear ethereal extract from the tubes. The measurement is most conveniently made with a pipette but care must be taken to keep the nozzle of the pipette under the surface of the fluid.

Note the amount of ether left in each tube.

Evaporate the ether extracts by holding the flasks in a stream of hot water.

Dry the residue by heating in an oven at 100°C . Unless this step is thoroughly done the error in the subsequent estimation is considerable.

After cooling weigh each flask.

The increase of weight of flask A represents the weight of total fat (neutral fat, free fatty acid and fatty acids combined as soaps) extracted from 0.25 gram of dried faeces supposing no ether to have been lost from the tube and consequently the level of the remaining fluid to be at the 30 c.c. mark. The percentage of total fat in the dried faeces is thus given by multiplying this increase of weight by 400. Similarly the increase of weight of flask B multiplied by 400 gives the weights of neutral fat and free fatty acid in 100 grams of dried faeces.

Dissolve the contents of B in 10 c.c. of alcohol, add 2 drops of phenolphthalein solution and titrate with alcoholic sodium hydrate until a permanent pink tinge appears. If x c.c. are required the free fatty acid in 100 grams of dried faeces reckoned as stearic acid is $400x \times 0.0284$ grams.

The neutral fat in 100 grams of dried faeces is the difference between the neutral fat plus free fatty acid and the free fatty acid thus estimated.

The fatty acid combined as soaps is the difference between the total fat and the neutral fat plus free fatty acid.

Normally dried faeces contain some 25 per cent of total fat of which neutral fat forms about half.

In 13 cases of carcinoma of the pancreas in which the faeces were thus analysed by Cammidge the total fat ranged from 50 to 90 per cent of the dried faeces and of this the neutral fat formed 40 to 60 per cent and the fatty acids 9 to 33 per cent.

An excess of fat sufficient to be of any significance can

usually be detected by examination of the dried fæces, without chemical examination. The application of this test is further discussed under pancreatic tests.

Mucin appears to be a normal constituent of the stools. It may be tested for by adding to a watery suspension of the stool an equal volume of lime water. The mixture is allowed to stand until the next morning and is then filtered. A little acetic acid is added to the filtrate and a white cloud of mucin, insoluble in excess of acid and increased on boiling, is produced.

Microscopical Investigation

Numerous points of considerable importance may be found in the course of a microscopic examination of the fæces.

In order to make the examination prepare in a test tube a turbid suspension of the fæces in normal saline. Shake thoroughly. Allow the suspension to stand. Pipette up the deposit and examine it on a slide with a cover slip over it in exactly the manner employed for a urinary deposit.

The general microscopic view of a fæcal suspension is at first confusing. The numerous particles of many varieties of food remnants and the general *debris* lying among the fauna and flora of the intestinal canal provide somewhat of a diagnostic debauch.

The following are among the substances which should be looked for —

Vegetable cells and fibres. These may occur in a great variety of shape and size. Spiral forms are extremely numerous, and may be found as free spirals or as long cells containing an evenly wound refractile spiral. Other forms appear as long and septate divisions marking off the usually empty cells. Some of the cells may contain chlorophyll and others starch granules.

Starch granules should not be present in great numbers, they may be detected by their appearance and by their blue reaction on running a solution of iodine and potassium iodide under the cover slip.

Fat. Fat globules should only be present in small numbers in a normal stool. They are present in large quantities after the injection of an oil enema. They are readily recognised by their shape and refractivity, and if necessary by their chemical reactions. Fat occurs more commonly in the form of sheaves

of colourless pointed crystals. These sheaves of fatty acid crystals dissolve on warming, and in ether. The soaps may appear in the form of coarser crystals and dissolve on warming but not on the addition of ether. When an excess of fat is present in the fæces fatty acid crystals are usually found, but a chemical estimation is advisable in order to determine whether fat is actually in excess or not.

Muscle fibres are nearly always found in the fæces of a person on a normal diet. Their amount and the degree of their digestion naturally vary with the amount of meat taken, but under ordinary conditions the relative number of undigested muscle fibres is a considerable guide to the activity of the digestive juice and the suitability of the diet to the individual. Muscle fibres as seen under the microscope are of a yellow colour and are certainly recognised by their fine transverse striation. The striation can be perfectly well seen with a $\frac{1}{8}$ inch objective particularly if the diaphragm of the condenser is partly shut down. Under normal conditions few fibres will be seen on any one slide and in the great majority of these the striæ will be almost or quite invisible. In conditions associated with alimentary disorders the fibres are very numerous and their striation well marked.

Elastic fibres can often be detected in the fæces, and are recognised by their shape, their curved form and their double contour.

Red blood corpuscles can as a rule only be recognised in the fæces under the microscope when the hæmorrhage has come from the lower part of the intestinal canal. With quite profuse hæmorrhage from high up in the gut, as in duodenal ulcer or even in typhoid fever, recognisable red cells are rarely met with. Reddish brown pigment masses are seen in such cases and can be recognised as of probable blood origin, but the chemical test for blood should always be applied. The sulphides of the metals and particularly of bismuth and iron, give an appearance to the stools similar to that produced by altered blood. Under the microscope the sulphides appear as black amorphous masses. Bismuth may occasionally appear in the stools in the form of black crystals of a shape similar to that of hæmin crystals.

Leucocytes are absent or almost completely absent, from the stools in health. In the numerous conditions associated with a catarrhal state of the intestines there is practically no

increase in the number of leucocytes found. Actual pus in the stools is very rarely seen, and any considerable increase in the leucocytes points to actual ulceration of the gut. Pus if present indicates that an abscess has discharged into the intestine, as for example an appendix abscess into the rectum. Pus cells have here, as elsewhere, to be differentiated from epithelial cells and in cases of doubt stained preparations should be made.

Epithelium Epithelial cells, more or less degenerated, are practically always found in the fæces. They may be squamous or less often columnar, and most commonly are fairly small more or less fusiform cells. They occur singly and less often in small plaques. In cases of intestinal catarrh epithelial cells are usually much increased in number.

Crystals The commonest inorganic crystal to be found is that of calcium oxalate, and the envelope forms are those most often seen. The crystals are derived from the food, and are most abundant after a vegetable diet. Other crystals which may be present are calcium carbonate and calcium sulphate rarely, calcium phosphate and triple phosphates more commonly. These crystals are recognised in the same way as in urinary deposits.

Bacteria are always present in considerable numbers in the stools. The majority of the organisms are bacilli, and in Gram stained films the Gram negative bacilli predominate in normal conditions, cocci are also present as a rule. Spirilla and vibrios are less common, but may appear in small numbers in health. The varieties of bacteria and their significance will be considered in the next chapter.

Ova of parasites are among the abnormal objects to be looked for in the stools. Their description and the method of examination is given on p. 164.

Amœbæ and the methods of detecting them in the fæces are described on pp. 151 and 152.

Débris Amorphous particles derived from the food form a considerable part of the slide preparations made from the fæces. They are of variable size, and may appear as discrete particles or in clumps.

CHAPTER XXII

THE PARASITOLOGY OF THE FÆCES

THE protozoa and animal parasites which may infect the intestinal tract are described in the section on parasitology (pp 151 to 164)

Bacteriology The fæces are normally so infested with bacteria that some departure from the ordinary routine bacteriological methods is necessary, and some clinical advice is particularly required as to the class of organism to be sought for. The presence of bacteria known to be pathogenic and to be normally absent from the intestinal tract such as the cholera vibrio or the typhoid bacillus is of diagnostic significance. The detection of organisms of atypical cultural characters and of doubtful pathogenicity should be amplified so far as possible by other methods such as the investigation of the agglutinating action of the patient's serum upon them and the findings, unless strongly supported by such means must be accepted with reserve. The detection of bacteria known to be found in the gut in health such as *B coli* has no diagnostic significance.

Film preparations of the fæces commonly give little bacteriological information but they should be made in the case of very liquid stools in order to gain an idea of the type of the prevalent organism. In cases of cholera the vibrio may be present in enormous numbers in the films. The cultural investigation necessarily varies with the type of organism looked for but in the majority of cases the following method will be found useful as a routine —

The fæces should be passed into an ordinary clean (not carbolised) bed pan and should be examined as soon as possible after they have been passed.

In an ordinary broth culture tube take 10 to 12 loops of the fænat or in a sterile platinum wire.

Leucocytes broth tube thoroughly without letting the fluid the stools in the wool plug and stand the tube aside for a with a catarrhal allow the solid particles to settle

Take a loop of the supernatant fluid and plate on 1 agar and 1 blood agar plate

Incubate the broth tube for from 4 to 6 hours at 37° C

Take a loop of the supernatant broth and plate it out on 3 MacConkey plates, without recharging the loop

Incubate the 5 plates till the next morning

Examine the agar plates for colonies of the staphylococcal and streptococcal type and for hæmolytic colonies

Examine the MacConkey plates for yellow colonies in particular

Take subcultures of the suspected colonies in the usual way

The aim of this process is to obtain plate cultures which are neither hopelessly overgrown nor contain merely one or two colonies, but which show a fair number of discrete colonies on the majority of the plates

The more important pathogenic organisms to be met with are the following —

The typhoid bacillus The bacilli can be isolated from the fæces by the above method, but it is preferable to make use of the fact that brilliant green has a greater antiseptic action upon *B coli* than upon *B typhosus*. A 1 per cent solution of brilliant green in distilled water is kept in stock, and, when required, a 1 per cent dilution of this is made in distilled water, 0.3 c c and 0.5 c c of this dilution are added to two 10 c c tubes of peptone water and incubated for 24 hours after inoculation with the fæces. If growth occurs in both tubes, that which contains the higher percentage of brilliant green is used for plating on MacConkey plates. The yellow colonies are picked off and tested by agglutination and cultural methods.

Paratyphoid bacilli These organisms are looked for by similar methods, but the brilliant green process is less successful with the paratyphoids than with *B typhosus*. In all cases the agglutinating action of the patient's serum and of known anti sera upon them should be investigated.

The dysentery bacillus Any of the types of dysentery bacilli previously described may be met with, and are to be sought for and identified by similar methods.

The cholera vibrio The recognition of the cholera vibrio is a comparatively simple matter in an epidemic, or in districts where cholera is known to be rife.

Sporadic cases of cholera are to be diagnosed with extreme

caution, since the differentiation between the genuine cholera vibrio and other vibrios which may occasionally be present in the gut is a matter of considerable difficulty

The examination of the fæces should be conducted as follows —

(1) Prepare a thin film of the fæces, and stain it with carbol thionin. In an acute case the organisms are usually present in considerable numbers, and tend to appear in groups, all the members of which lie with their long axes in the same direction, as trout lie in a stream

(2) Put several loops of the fæces into broth, and incubate for about 4 hours

Plate from the broth on to 3 gelatin plates, and incubate at from 18° to 20° C for 24 hours

Examine the plates for colonies of the vibrio. These colonies are white with jagged outlines, and have the appearance of powdered glass on the surface of the plate. Film preparations from them show the vibrio

(3) Subculture from the gelatin plate into broth, on to agar slopes, and in gelatin stab cultures. Continue the incubation of the gelatin plate

After incubation for from 24 to 48 hours of the broth culture add to it a few drops of pure sulphuric acid. A rose pink colour of nitroso indole develops in the medium

The agar slope culture shows a yellowish irregular slimy growth

The gelatin stab culture shows a white streak of growth along the track of the inoculating wire, and at the surface of the medium a funnel shaped depression of commencing liquefaction

The colonies on the gelatin plate slowly darken, and liquefaction takes place in the medium

(4) Test the agglutinating action of a known anti serum upon the culture

(5) Test the lytic action of an anti-serum on the culture *in vitro* by incubating the vibrios, the serum and fresh guinea pig serum in a hanging drop for from 1 to 2 hours. The same test performed *in vivo* by injecting broth and anti serum into the peritoneal cavity of a guinea pig is known as Pfeiffer's reaction. Tests for lysis are not essential and have been largely superseded by the agglutination reaction

(6) Inoculation of the cholera vibrio into the peritoneal

cavity of a guinea pig produces a typical toxic effect Other vibrios are non pathogenic to animals

In the presence of an epidemic microscopic and cultural examinations are sufficient for diagnosis but in all other cases the agglutination reaction must be carried out

Among the organisms liable to be confounded with the vibrio of Asiatic cholera are the following —

The vibrio of cholera nostras described by Finkler and Prior may be found in considerable numbers in the stools of patients suffering from acute diarrhoea and vomiting such as may occur in temperate climates in the summer months In the majority of such sporadic cases however vibrios in the stools are either very scanty or absent The Finkler Prior vibrio is longer and broader than Koch's vibrio and on gelatin plate culture forms round colonies with sharply cut edges Gelatin is liquefied rapidly

Similar vibrios which may be found in the stools of healthy persons or patients with intestinal affections have been isolated from cheese and from the water supplies of towns Some of these vibrios closely resemble the cholera vibrio both on morphological and cultural grounds and can only certainly be distinguished by serum tests

Tubercle bacillus This organism may be present in the faeces in large numbers in cases of tuberculous enteritis and in such cases pus is present in addition and often blood The bacilli are present in small numbers in the case of persons with pulmonary tuberculosis and particularly of children who are more likely to swallow the sputum

The faeces should be treated with antiformin and the bacilli looked for in the ordinary way

Other organisms Among others of the commoner organisms to be met with in the normal faeces are staphylococci streptococci and less commonly *B. pyocyaneus* and several of the anaerobic bacilli including *B. tetani* and *B. Welchii* Cocci are commonly present and the streptococci met with are as a rule of very low pathogenicity for animals In cases of ulcerative colitis the organisms commonly suspected and looked for as the causative agents are members of the coli typhoid group and it is quite possible that coccal infection may in some cases be overlooked

The cultural examination of colitis is aided by the passage of the sigmoidoscope After thorough irrigation of the colon

with sterile tap water a minute portion of the infected mucous membrane can be safely removed by a skilled operator and transferred to a culture tube. By such means streptococci may be found in pure culture in a small percentage of cases, and a vaccine prepared from the organism may be beneficial.

The isolation of cocci from fæces by the ordinary methods may be difficult and can have no particular significance.

SECTION VI.—THE EYE AND SKIN

CHAPTER XXIII

THE EYE AND CONJUNCTIVAL SAC—THE SKIN

THE EYE AND CONJUNCTIVAL SAC

THE cytology of the conjunctival sac does not materially differ from that of other parts of the body. In the more chronic infections of the conjunctiva large epithelial cells are commonly met with, and in the acute inflammations the ordinary polymorphonuclear cells of suppuration. In the rare condition known as "spring catarrh" the exudate consists almost entirely of eosinophil cells.

The bacteriology of the eye is of considerable importance, and in a variety of affections much assistance is obtained from a careful bacteriological investigation.

The normal conjunctiva, being exposed to air contaminations, is rarely sterile, and some acquaintance with the organisms present in health is necessary. The bacillus known to ophthalmologists as the *xerosis bacillus* is the organism most frequently met with, and is present in the majority of cases examined. The *xerosis bacillus* is no longer believed to play any essential part in the production of the condition the name of which it bears. It is a diphtheroid organism, and will be subsequently referred to under that name. The diphtheroid bacilli met with in the eye are of more than one variety, but the great majority of them belong to a type which grows readily on the ordinary solid media, such as agar or blood serum, but merely maintains its existence in broth and other liquid media. It does not acidify litmus dextrose broth. Next to the diphtheroid bacilli a white staphylococcus of low virulence is the organism most frequently met with. Exceptionally, more virulent organisms, such as *staphylococcus aureus*, *streptococcus hæmolyticus* and the *pneumococcus* may be cultivated from the apparently normal conjunctiva.

The occasional finding of virulent organisms in normal cases is of considerable importance, and it is a reasonable precaution to make a bacteriological examination in all cases before conducting such an operation as that of cataract extraction. It is an essential precaution if any inflammatory condition of the lids or conjunctiva is present, however mild.

Conjunctivitis The commonest and most widely spread variety of acute conjunctivitis is that due to the *Koch Weeks bacillus*. The disease has a short incubation period of about 24 hours, is extremely contagious, begins as a rule in one eye and almost invariably spreads to the other, is associated with redness and swelling of the lids and conjunctivæ and runs a variable course often lasting from 2 to 4 weeks. The diagnosis can be sufficiently confirmed by film preparations. The minute, slender Gram negative bacilli exactly resemble in appearance the influenza bacillus (p. 115).

Diplo-bacillary conjunctivitis The causative organism of this disease is known as the *Morax Arenfeld bacillus*. The condition is widely distributed, and is more chronic than the Koch Weeks infection. It is infectious, almost always affects both eyes, and is associated with a characteristic redness of the angles of the palpebral fissure. The infection may in untreated cases last for years. A nasal catarrh is sometimes present in addition, and the causative organism may be found in the nasal discharge. In films made from the pus the bacilli are found as stout rods of moderate length with rounded ends, the great majority of them being in pairs and outside the cells. Occasional chains are present. The occurrence of Gram negative bacilli of this nature in film preparations is sufficient to establish a diagnosis. A growth of the bacilli in the form of small, translucent colonies on serum agar can often be obtained.

Gonorrhæal conjunctivitis has been already referred to in the description of the gonococcus. The causative organism is as a rule, numerous in the conjunctival secretion, and the condition is readily recognised in film preparations.

Trachoma The cause of this disease is unknown, and numerous parasites, subsequently discredited, have been from time to time described. Noguchi described a small Gram negative bacillus, with which he was able to produce a trachoma like condition in monkeys. The etiology of the disease is still, however, *sub judice*.

Other varieties of conjunctivitis A purulent conjunctivitis may be set up by any of the ordinary pyogenic bacteria and the investigation of the exudate should be conducted on the ordinary lines. In no case should a diagnosis of the organisms be made from film preparations alone. Mixed infections are not infrequent. In a series of investigations dealing with this class of infection the *staphylococcus albus* was found twenty eight times *staphylococcus aureus* twenty seven times a *streptococcus* ten times an *intermediate staphylococcus* three times the *diphtheria bacillus* twice and an unclassified coccus once. *Diphtheroid bacilli* were also present in about 60 per cent of the cases. In addition to these organisms the *pneumococcus* may exceptionally be found as the cause of a primary conjunctivitis and it has been described as a common cause owing to the improper identification of the organism by film preparations only. Epidemics of conjunctivitis due to the pneumococcus have been recorded. The diphtheria bacillus is a rare cause of conjunctivitis being usually met with in children and in association with a nasal discharge containing the same organism. The diphtheria bacillus should be identified in this situation not only by its morphological and cultural characters but also by animal inoculation. Diphtheritic infection of the conjunctiva is associated as elsewhere with membrane formation and is readily amenable to serum treatment.

Membranous conjunctivitis is less commonly due to the diphtheria bacillus than to other organisms of which the most important is the *streptococcus hæmolyticus*. Streptococcal infection of the conjunctiva is the most virulent type met with and a small percentage of cases proceed rapidly to panophthalmitis in spite of all treatment. Less commonly staphylococci may produce a membranous conjunctivitis.

The lids The bacteriology of the lids is practically identical with that of the skin and all the commoner varieties of inflammation associated with the pyogenic cocci are met with. *Molluscum contagiosum* is occasionally encountered here as elsewhere on the exposed surfaces.

The lachrymal sac Inflammations of the sac are set up by the ordinary pyogenic organisms of which a *streptococcus* is perhaps the most common. Here again *pneumococci* may be present but have been described more frequently than is probably correct owing to an undue reliance upon film prepara-

tions The *micrococcus catarrhalis* is another organism occasionally associated with this condition

The cornea The main organism concerned in the production of the serpiginous corneal ulcer is the *pneumococcus* The infection is, however, not a truly specific one, and other organisms, such as the staphylococci and streptococci, may be present in pure culture Bacilli of the *colon* group, *B proteus* and *B pyocyaneus*, are occasionally found in corneal ulceration, as well as in exceptional cases of conjunctivitis without corneal infiltration *Aspergillosis* of the cornea has also been recorded on numerous occasions

The chronic infective granulomata *Tuberculosis*, *leprosy* and *syphilis* may all affect the eye The methods of recognising the causative organism are the same as those adopted for these diseases in other parts of the body

Endogenous infections Metastatic abscess in the eye may arise in the course of a general infection produced by any of the pyogenic organisms An interesting variety of metastatic ophthalmitis occurs in epidemics of cerebro spinal meningitis, and a similar condition, producing a disease known as "*pseudo glioma*," has been demonstrated to be caused by the *meningococcus* and to occur in cases with no history of meningeal symptoms The coccus has been recovered from the interior of the eye after excision

The orbit The bacteriology of acute inflammatory conditions of the orbit calls for no particular description Infection of the orbit arises by direct extension from adjacent parts in the great majority of cases The most important sources of infection are the accessory sinuses of the nose

THE SKIN

There is scarcely any pathological investigation which may not from time to time be required for patients whose main complaint is of some skin lesion The following are among some of the changes more particularly associated with diseases of the skin —

The blood An *eosinophilia*, often of marked degree, is frequently associated with many widespread dermal lesions In the specific fevers associated with skin eruptions, and particularly in small pox, chicken pox and scarlet fever, a considerable eosinophilia is the rule In the two former

diseases the eosinophils diminish in number and finally disappear when the bullæ suppurate. Among other diseases associated with a vesicular eruption dermatitis herpetiformis is almost constantly accompanied by a considerable eosinophilia. In films made from the bullæ in these cases, as well as in small pox and chicken pox numerous leucocytes are present, and the great majority of them are eosinophils. In pemphigus chronicus, on the other hand, eosinophils are absent from the blebs, and there is no eosinophilia in the blood. The cytological character of dermal exudates is thus of some assistance in differential diagnosis. Secondary infection of skin vesicles, however, very readily occurs, and it is necessary to examine the fluid as soon as possible after the vesicle has appeared. Cases are not infrequently met with in which vesicles have been deliberately produced by the patient either by the aid of blistering fluid or some cruder device. In these mechanical effusions the great majority of cells present are of the epithelial type, and the presence of such cells is strongly suggestive of an artificial lesion.

Leukæmia is very rarely associated with considerable leukæmic infiltrations of the skin. These infiltrations have, however, on occasion been so considerable as to merit the name of "tumours," and the patients have first come under the observation of a skin clinic. Rare as the condition is, it is advisable in all cases of multiple skin tumours of doubtful origin to make an examination of the blood. The blood changes present are usually those of myeloid leukæmia.

Pernicious anæmia is nearly always accompanied by a lemon yellow colour of the skin, and very exceptional cases are met with in which the pigmentation is so extreme as to produce an almost negroid coloration. In the condition usually described as hæmochromatosis, and often associated with diabetes mellitus, there are no specific blood changes. In certain rare cases of erythrodermia the patient has the colour of a Red Indian (*homme rouge*) and the characteristic blood changes previously described (p. 19).

Syphilis. An examination of the Wassermann reaction in the serum is frequently required, and should be performed in all cases of syphilitic disease with skin lesions as well as for patients with rashes of a doubtful nature. The spirochæte should also be looked for in the primary sore, and may be found in the condylomata and other secondary lesions.

The parasitology of skin diseases The skin is peculiarly liable to infection both by animal and vegetable parasites and a complete description of all such organisms as may be found in the skin can only be given in a book devoted to dermatology. A brief account only of the more important animal parasites has been given in a previous section (p. 143).

Bacteria The normal skin The normal skin like the normal conjunctiva being an exposed surface is in consequence rarely sterile. The organisms to be met with on almost any skin and in almost any part of the body are white staphylococci, sarcinæ and diphtheroid bacilli. These organisms are under ordinary circumstances non-pathogenic. It is possible that they play some part in the normal metabolism of the skin and that in diseased conditions they may even become pathogenic. The number of these organisms present on the skin of different individuals varies considerably and they are a common source of contamination in culture tubes whether derived from the defective technique of the bacteriologist or from the skin of the patient. The diphtheroid bacilli and sarcinæ are readily recognised and their presence in culture tubes may be regarded as evidence of contamination. The staphylococci of the normal skin are almost always represented by a white variety which is very inactive in culture media growing readily but producing little change.

Less commonly more virulent organisms may be grown from the normal skin and the majority of pathogenic species can on occasion be obtained. *Staphylococcus aureus* or *citreus* and the *streptococci* are the most important organisms met with and were of particular importance to the surgeon before the introduction of rubber operating gloves. They are still of importance if present on the skin of the patient. Virulent organisms can maintain their existence on the skin without producing any harmful results upon their host. They may exist for considerable periods in spite of attack by all the ordinary methods of cleanliness including antiseptics. The action of antiseptics applied to the skin rarely succeeds in destroying all bacterial growth without injury to the epidermis. A minute trace of a powerful antiseptic however will inhibit or delay the subsequent growth of organisms and in cultures taken from a focus which has been exposed to antiseptic action it is common to find the visible growth of a freely multiplying species delayed by 2 or 3 days.

Streptococcal infections of the skin are common, and the most notorious infection produced is *erysipelas*. Erysipelas proper is an acute inflammation of the dermis, and the organisms spread along the superficial layers of the skin, but cultures made from recent bullæ are in a considerable percentage of cases sterile. If cellulitis is present in addition, and incisions are made, the streptococci can be obtained in pure culture. The local lesion in erysipelas may provide the causative organism, but in cases of facial erysipelas particularly the local lesions may not be obvious, or may be situated in the nasal cavity and consequently associated with numerous other bacteria.

Impetigo contagiosa is a primary infectious disease most often met with in children, and produced by the *Streptococcus pyogenes*. The secondary impetigo caused by scratching in patients affected with scabies, pediculosis, or other irritative lesions may be streptococcal, or more commonly staphylococcal, mixed infections are, however, frequent. The follicular impetigo of the hair follicles is essentially a staphylococcal disease.

Pemphigus neonatorum is usually associated with a septic condition of the umbilical cord stump and is a disease attended with a high mortality. The causative organism may be a streptococcus or a staphylococcus.

Staphylococcal infections of the skin are, as might be expected, extremely common, and very varied lesions are produced. *Follicular impetigo* of the skin and of the scalp is a frequent infection, particularly among ill cared for children. The condition may be transmitted by one child to another, is liable to relapse, and often runs a prolonged course. The causative organism is nearly always the *Staphylococcus aureus*, but mixed infections are not infrequent. The lesions are readily inoculated by the patient from one part of the body to another. Staphylococcal inflammations of the eyelids or conjunctivæ may be produced by auto inoculation from an impetiginous focus on the finger or the face.

Boils are sequels of follicular impetigo, and due to the same organisms. They are prone to occur in the debilitated as well as among those in particularly vigorous health. *Carbuncles* are a more serious lesion produced by staphylococci, of which *Staphylococcus aureus* is the most frequent agent, but mixed infections occur and *S. hæmolyticus* is not infrequently associated with the staphylococcus.

Multiple *subcutaneous abscesses*, due to staphylococcal infection, are sometimes met with, and are not uncommon as a sequel to prolonged fevers. Typhoid fever is fairly often associated in the post febrile stage with the appearance of numerous and widely-distributed subcutaneous abscesses. The *Staphylococcus aureus* or *albus* is nearly always obtained in pure culture, and we have not found the typhoid bacillus in these lesions. Subcutaneous abscesses may result from a general blood infection and be associated with pus formation in other parts of the body, but in the majority of cases the patient's general condition is good, and the abscesses appear to result rather from the local atrophic condition of the skin following fever and the prolonged stay in bed. The production of bed sores is a similar process in which pressure plays a prominent part.

Whitlow is often of staphylococcal origin, and when accompanied by a spreading lymphangitis is practically always due to the *Staphylococcus aureus*.

Barber's rash, or *sycosis*, is one of numerous local skin infections, many of which are provided with special names, and the majority of which are due to staphylococci. *Seborrhœa* may be produced by a variety of organisms, including staphylococci, and in view of the extraordinary septic habits of many barbers it is remarkable that more virulent infections are not frequently transferred from one victim to another. Fortunately the highly priced "tonics" applied after the operation usually contain some cheap and useful antiseptic.

Treatment In the treatment of all staphylococcal infections of the skin surgical measures should be as conservative as possible, and some form of bacterial therapy is usually advisable. Staphylococcal infections tend to recur, and in many cases recurrence can be prevented by vaccine therapy, and this must be undertaken with care, since it is possible to increase by vaccines that state of increased sensitivity to the organism which is induced by the infection itself. The vaccine given should rather be one derived from a vigorous toxin producing strain than the actual infecting strain, should this be a poor toxin producer. The dose employed should be one which gives little or no local reaction, and it is advisable to limit the length of treatment, if possible to a few weeks, and to stop it at once if an injection of vaccine appears to be followed by a fresh lesion. The toxin, or preferably toxoid, can be given

instead of a vaccine, and in many cases immunisation by toxoid is the method of choice

The toxin can be easily prepared by growing the organism on agar for 48 hours, grinding up the culture media and growth in sterile saline and filtering through a bacterial filter (p 237) The filtrate should contain hæmolysin for rabbit or sheep cells in high titre 0.3 per cent formaldehyde is added to the toxin, which is then incubated at 37° C for 3 days, or until the hæmolytic action has disappeared Treatment should commence with very small doses since the local reaction may be severe An initial dose of 0.5 c.c. of a 1 in 60 dilution of toxoid is usually suitable

Acne vulgaris is a disorder of the sebaceous glands, commencing as a rule, at puberty and rarely lasting beyond the twenty fifth year A small Gram positive diphtheroid organism the acne bacillus can often be isolated from the comedones before suppuration occurs but there is little real evidence that it plays any part in the etiology of the condition Vaccines made from the acne bacillus have been largely employed, and probably owe their popularity to the frequency with which acne tends to spontaneous recovery When, as often happens acne is complicated by suppuration due to secondary staphylococcal infection, treatment by staphylococcal vaccine or toxoid is often of benefit

Anthrax is a rare infection occurring among hide porters, wool sorters and butchers An account of the anthrax pustule and of the bacillus has been given in a previous chapter (p 110)

Other pyogenic organisms which may affect the skin are numerous but the majority of such infections are not primarily dermal and spread to the skin by extension from the deeper tissues

Tuberculosis of the skin assumes many clinical forms, and on a pathological basis may be divided into at least two varieties In one form the tubercle bacilli are present in the lesion, although as a rule in very small numbers The bacilli are, in a considerable percentage of cases, of the bovine type, and the lesions which they produce have to be recognised mainly from their clinical features Material is not ordinarily available for bacteriological examination, and the diagnosis has to be confirmed when necessary by removal of a portion of skin The histological evidence of tuberculosis can sometimes be confirmed by detecting the bacilli in the sections,

but the organisms are commonly very scanty, and often proof can only be completed by inoculation of portions of tissue into guinea pigs. Diagnostic injections of tuberculin are nearly always positive, and a negative result is strong evidence against tuberculosis.

In the other class of tuberculous infections the bacillus appears to be entirely absent from the lesion, and to such conditions the term "tuberculide" is applied. It is supposed that the affections may be caused by the toxins of the bacillus which have been absorbed from a distant focus.

Leprosy is characteristically associated with the formation of granulomata in the skin. The bacilli are numerous in the lesions and in the discharges from them, and the diagnosis can nearly always be confirmed by the examination of swabs taken from the nasal secretion. The *lepra bacilli* are often present in this situation in large numbers.

Syphilis and the means of detecting the *Spirochæta pallida*, together with the importance of the Wassermann reaction, have already been considered.

Another disease affecting the skin and produced by spirochætes is *Yaws*. This disease has some clinical features in common with syphilis, and is produced by a spirochæte extremely like the *S. pallida*.

SECTION VII.—THE RESPIRATORY TRACT

CHAPTER XXIV

THE NOSE—THE SPUTUM—BASAL METABOLISM

THE NOSE

THE examination of the nasal secretion is mainly bacteriological, and since cultures taken from the nasal cavity are practically never sterile, the results of bacterial examination in disease must be critically considered. The methods of bacterial investigation do not materially differ from those previously described.

It is advisable as a routine to use a cotton wool swab similar to that employed for diphtheria cultures, and to plate direct from it on to two blood agar plates. A further culture may also be made into broth, and film preparations should be made in addition in all cases.

The following are among the affections which may especially require investigation —

Nasal catarrh The usual attack of nasal catarrh is commonly left to run its course with or without the aid of domestic remedies. Some persons are so unfortunate as to suffer from repeated attacks at short intervals, and particularly during the autumn and winter months. Vaccine treatment has been very largely employed to meet such cases. There is now little doubt that the common cold is due to a filterable virus, but the prolonged and purulent nasal discharges which may follow are almost certainly due to secondary infection by pyogenic bacteria, and from these vaccines can reasonably be prepared. Provided there is no local nasal condition such as can be rectified by the surgeon, and the patient is seriously embarrassed by the attacks, the causative organisms should be investigated. The attacks of nasal catarrh are often preceded or accompanied by tonsillitis, and it is preferable to swab the tonsil and, by means of a bent swab, the retro-pharyngeal space as well as the nasal cavity. West's swab is

a convenient apparatus for taking cultures from the retro-pharynx or from any deeply situated space such as the interior of the uterus. A length of glass tubing is bent to a convenient angle at about $1\frac{1}{2}$ inches from its distal end. A wire with cotton wool swab attached is inserted along the tube to just beyond the angle. The glass tube is plugged with wool and the whole is sterilized. When using the swab remove the plug from the tube pass the tube behind the palate, press the swab forward and beyond the tube to take the culture, withdraw the swab again into the tube and then remove the tube. The swabs can be plated out and the bacteriological results compared. In many cases recurrent winter colds are prevented by a course of 8 to 10 injections of vaccine, preferably given in the early autumn. It is usually advisable to repeat the course in the following year. A considerable percentage of cases derive no benefit.

The organisms which may be met with include the *micrococcus catarrhalis*, *streptococci*, *staphylococci*, and less commonly *pneumococci* and *Friedlander's pneumobacillus*. The vaccine is preferably made from the predominant and most virulent organism. The selection of the causative bacterium may be a matter of chance, and a mixed vaccine can be employed.

Hay fever. A standardized vaccine of pollen toxin may be given for this condition or the skin sensitivity to the various pollens may be tested. It is probable that all cases of hay fever are not due to pollens. Many are associated with asthma, and some are apparently of bacterial origin and accompany what appear to be ordinary attacks of nasal catarrh. The treatment by pollen extracts or vaccines can be made on the same lines as for the common colds, and the value of the treatment is much the same in the two conditions. Pollen extracts for test purposes and for treatment can be purchased. A less specific, but often more successful therapy, consists in the administration of a series of graded doses of peptone. Both in the treatment of asthma and hay fever the special peptone injections advocated by Auld may give excellent results. Thus peptone is put up in a series of graduated doses by Martindale.

Diphtheria. Nasal diphtheria is not infrequently met with in children, and the bacteriological examination is conducted in the same manner as in tonsillar diphtheria. Harmless

diphtheroid bacilli, however, are more commonly met with in the nose than in the throat, and in cases of clinical doubt it is advisable to inoculate a guinea pig with a culture of the suspected organism

Leprosy The examination of the nasal secretion in cases of leprosy has already been considered. The bacilli are almost *invariably present in the nose in considerable numbers and* in the early stages of the disease. There may be little actual discharge noticeable to the patient and the secretion is often thick, crusted, and difficult to manipulate

THE SPUTUM

General examination of the sputum *Naked eye observations* The *amount* of the sputum is of importance in certain diseases, and in particular in cases of bronchiectasis and pulmonary tuberculosis. The amount may vary from a few cubic centimetres in the early morning to as much as a litre in the 24 hours

The *odour* is practically inoffensive in the majority of affections, but may be quite overpowering. Bronchiectatic sputum is nearly always offensive as is also that from an abscess of the lung. If gangrene of the lung is present the odour is indescribable

The *colour* is whitish in early and mild catarrhal cases. It becomes yellow with advancing suppuration. It is red if blood is present. Blackish particles visible to the naked eye, are nearly always present, and are due to inspired atmospheric carbon

The *consistence* and general appearance give some guidance. The sputum in pneumonia is particularly viscid and blood streaked, and in tuberculosis of the lung the sputum may have a "nummular" character. Fibrinous casts, spirals and shreds of solid tissue may in certain conditions be visible to the naked eye

Microscopical examination The sputum can be examined microscopically both in the fresh state, by squeezing a portion between a slide and a cover slip, and in stained film preparations

The stained films show in the majority of cases large epithelial cells and leucocytes of the polymorphonuclear variety in varying proportions, fibrinous strands, and large numbers of

organisms The structures to be examined for are the following —

The cells Epithelial cells are nearly always present, but if they form the great majority of the cells in a film the "sputum" probably comes mainly from the mouth and upper air passages Tubercle bacilli are rarely found in such specimens, which are liable to be produced by a patient to order and to be examined, with the misleading result that tubercle bacilli are stated to be absent

Pus cells are present in all sputa whether the underlying condition is tuberculous or not

Eosinophils are not recognised in carbol thionin or methylene blue preparations, they must be specially stained for, and are well seen in thin films treated with Leishman's stain in the ordinary way Eosinophils may form the predominant cell in cases of genuine spasmodic asthma

Red blood corpuscles may be recognised in the fresh specimens and in the stained films They stain a greenish colour with carbol thionin, but are best fixed and stained by one of the blood stains

Elastic fibres Before the discovery of the tubercle bacillus the presence of elastic fibres in the sputum was regarded as of great diagnostic importance The fibres are now rarely looked for, but their presence in sputum is of significance, since they indicate that there has been actual destruction of lung tissue Elastic fibres in small numbers may find their way into the sputum from the food, and it is advisable to instruct the patient to cleanse his mouth thoroughly before obtaining a sample of sputum

Elastic fibres are found in tuberculosis, bronchiectasis, and pulmonary abscess They are occasionally met with in lobar pneumonia apart from abscess formation In actual gangrene of the lung the fibres are rarely found, probably because they have been dissolved locally by ferment action

Single elastic fibres are difficult to detect and are of little diagnostic importance, since they may have been introduced in the food, unless very particular care has been taken Elastic fibres occurring in bundles which display an alveolar arrangement are more readily detected, and certainly come from the lung

The fibres vary in size, and have a wavy outline and double contour If they are present in considerable numbers it is only

necessary to mix a little sputum on a slide with 10 per cent. caustic potash, and to spread it out under a cover slip. The fibres are more resistant to the potash than the other constituents of the sputum, and stand out as curved refractile threads.

Curschmann's spirals These spiral bodies are found in great numbers in the sputum of cases of spasmodic asthma. They are not found in asthmatic cases of old standing in whom advanced emphysema and bronchitis have occurred. The sputum in such cases is of the ordinary bronchial type. The spirals are present in the sputum which immediately follows a true spasmodic attack. They are not confined to asthma, but may occasionally be observed in cases of acute pulmonary tuberculosis.

The spirals are visible to the naked eye, and appear as white twisted tenacious bodies in the sputum. Examined under the microscope they are seen to consist of a coarse central thread round which is wound a twisted meshwork of delicate fibrils. Their source of origin is probably the small bronchioles.

Charcot-Leyden crystals These are colourless elongated, and sharply pointed octahedral crystals. They are insoluble in water, alcohol or ether and soluble in acids and alkalis. They are frequently found in the sputum, particularly after standing, of asthmatic patients but are not diagnostic of this disease, and probably are of no particular significance.

Fibrinous casts Small fibrinous casts of the finer bronchioles are occasionally detected in the sputum in lobar pneumonia, broncho pneumonia and rarely in bronchitis. They are whitish in colour, and of moderately firm consistence. Large fibrinous casts are practically confined to the very rare condition known as fibrinous or chronic plastic, bronchitis. The fibrinous coagulum consists of a branched stem with numerous subdivisions, resembling the leafless branch of a tree. Almost the complete cast of a bronchial system may occasionally be expectorated.

Asbestos bodies are found in the sputum of asbestos workers and are evidence of exposure to asbestos dust, but not necessarily evidence of fibrosis of the lung. The bodies are developed by the deposit upon fine asbestos fibres of an iron containing material formed in the tissues. They are examined for by digesting the sputum with an equal volume of antiformin and

centrifuging the washed deposit. In unstained preparations they form very striking objects with a considerable diversity of shape and occur either singly or in clusters. Of a golden yellow colour they vary in length from 24 to 60 μ or more and in shape according to the coarseness and distribution of the deposit upon the central fibre.

The sputum in various diseases. *Bronchitis* In the earlier stages is accompanied by a whitish viscid and scanty sputum which later becomes yellow, copious and obviously purulent. The sputum is rarely nummular but cannot be distinguished by its appearance from that of tuberculous cases.

Bronchiectasis The sputum is copious and is usually brought up in large quantities at a time with considerable intervals between the attacks of expectoration. It is as a comparatively fluid and almost invariably has a highly offensive odour.

Pneumonia At the onset of lobar pneumonia the sputum is very scanty or absent and in exceptional cases there is very little sputum throughout the course of the disease. Later the sputum becomes abundant and is characteristically tenacious. Owing to the intimate admixture of the blood exudation in the pulmonary tissues the sputum is more or less evenly blood stained and is commonly described as rusty in appearance.

Pulmonary tuberculosis In the majority of cases there is little in the appearance of the sputum by which tuberculous cases can be distinguished from other pulmonary conditions. The presence of blood in the sputum but not intimately mixed with it as in lobar pneumonia is always suggestive of tuberculosis. The blood is bright red as a rule and may be in large amount or the sputum may be streaked with blood. Nummular sputum is also indicative of tubercle of the lung with cavity formation. The nummular character is best seen by floating the sputum in water when the round more or less flattened discs of mucus separate out and finally sink to the bottom. The only valuable evidence of tuberculous sputum is the finding of the tubercle bacillus. The bacilli are to be found in the very great majority of all cases of pulmonary tuberculosis associated with a considerable degree of lung rectoration.

The *enzia* is associated in the early stages with sputum of contourary bronchial character and in the later stages often

becomes extremely profuse and very tenacious. The bacilli can be detected as a rule in ordinary film preparations.

Asthma In cases of spasmodic asthma the sputum commences as the dyspnoea passes off, and small characteristic pellets are coughed up. The pellets contain eosinophil cells in large numbers, Curschmann's spirals, and often Charcot-Leyden crystals.

Abscess In abscess of the lung the sputum may consist of pure pus with practically no mucoid admixture. In cases of any standing the smell is always offensive. The abscess may come from the lung itself or from the pleural cavity after rupture of an empyema into the lung. The material expectorated may be indistinguishable from the contents of a bronchiectatic cavity. In all such cases numerous fine, long bacilli are present and are probably responsible for the odour.

A tropical abscess of the liver may rupture into the lung and give to the sputum an appearance of having been mixed with anchovy sauce. Amœbæ may be detected in the sputum.

Gangrene is associated with sputum of a green colour and extremely offensive odour. Gangrene of the lung may follow an injury, and is the rarest sequel of lobar pneumonia.

Malignant disease of the lung is usually associated with hæmorrhagic sputum. The expectoration has been pleasantly compared to red currant jelly.

Malignant disease of the œsophagus is very commonly associated with a very profuse pale, watery, and tenacious secretion. The occurrence of this material in the sputum pot is strong evidence of a malignant, as opposed to a spasmodic, stricture of the œsophagus.

Edema of the lung is accompanied by a copious white, frothy sputum. When blood is present in addition the expectoration is commonly likened to prune juice.

Infarction of the lung is accompanied by a bright red sputum intimately mixed with froth.

Pneumonoconiosis results in a brownish black sputum. The colour is due to particles of carbon in "anthracosis," or of sulphide of iron in "siderosis," or to lime dust in "stone-mason's lung."

The parasitology of the sputum Infection of the lungs by the higher animal parasites is not common. In hydatid disease of the lungs or pleura the characteristic hooklets can

sometimes be found in the sputum, and small cysts may be coughed up entire. In cases of endemic hæmoptysis met with in China and other parts of eastern Asia the ova of the *Paragonimus westermani* (p. 154) are to be looked for in the sputum.

The amœbæ of dysentery, after rupture of a liver abscess into the lung, are looked for in the sputum in exactly the same manner as in the fæces (p. 151). Failure to find the amœbæ is not uncommon. The organisms are more likely to be met with 2 or 3 days after the rupture of the abscess has taken place.

Bacteriology of the sputum. In almost every pulmonary affection the sputum swarms with a variety of organisms and, apart from the detection of the tubercle bacillus, the recognition of the causative bacterium is usually a matter of considerable uncertainty.

The following procedure may be followed for the routine bacteriological examination of the sputum —

(1) The mouth should be cleansed as far as possible before the material is expectorated, but strong antiseptics must be avoided in the cleansing.

(2) A sterile test tube fitted with a cotton wool cork and a sterile glass filter funnel is provided, and the patient is directed to remove the cork from the tube to insert the funnel and to expectorate down the funnel into the tube. The funnel is then removed and the tube re-corked.

(3) Elaborate methods of washing, filtering and teasing the sputum are in use, but perfectly satisfactory results are obtained by simply taking a loop of the sputum in a sterile platinum wire, and plating it out on two blood agar plates without re-charging the loop. Discrete colonies are almost invariably obtained in this way.

A broth culture is preferably put up as a control at the same time.

Film preparations are also made.

(4) Incubate the plates till the following morning.

(5) Examine the films and the plates for the predominant organisms, and for hæmolytic bacteria.

Remove with the platinum wire sample colonies from the lung sputum culture them.

The *enzas* are from the secondary cultures into the contourary bacteria organisms likely to be of pathological

importance Ignore colonies of sarcinæ, spore bearing bacilli, and in most cases staphylococci

Among the organisms to be looked for in the sputum are the following —

The tubercle bacillus The methods of examining the sputum for tubercle bacilli have already been described (p 226) If there is any urgency films may be made from the sputum direct, and stained by the Ziehl Neelsen process In the majority of cases it is preferable to carbolise the sputum and let it stand till the next day In those cases in which tuberculosis is strongly suspected and there is an appreciable quantity of sputum, yet no tubercle bacilli have been found by the carbolic acid process, a further sample of sputum may be treated by the "antiformin" method It is, however, exceptional to detect bacilli in a sample of sputum after treating with antiformin if they have not previously been found after carbolising

In all doubtful cases at least 15 minutes should be given to each slide before abandoning the search, and it may be necessary to examine the morning sputum on two or three occasions

If the patient with suspected pulmonary tuberculosis is a child who swallows the sputum it is worth while to examine the fæces for the bacilli by the antiformin method, or the gastric washings (p 226)

The pneumococcus In films made from typical pneumonic sputum the characteristic encapsulated diplococci may be present in great numbers, but the exact recognition of the causative organism requires further investigation

Direct typing of the pneumococci may usually be made from sputum with the help of antisera The method has been described on p 239

While the majority of cases of lobar pneumonia are produced by the pneumococcus, a substantial minority are due to streptococci and other organisms Streptococcal pneumonia is commoner in children than in adults, and more often has a broncho pneumonic than a lobar distribution

Friedlander's pneumo bacillus This organism is present in the sputum in some cases of pneumonia, as well as in other conditions It may be found also in the mouth and in the nasal cavity

The bacilli are Gram negative capsulated organisms, which

usually appear as short rods, with rounded ends. They occur in the films in small groups of 2, 3, and 4.

The pneumo bacillus is best isolated from the sputum by plating direct on two gelatin plates. The colonies appear as white, heaped up points on the plates, and there is no liquefaction of the gelatin. In gelatin stab cultures growth occurs along the track of the inoculating wire, and profusely in a round, heaped up growth at the surface of the stab. The growth in gelatin stab cultures is described as "nail shaped." Litmus milk is acidified and clotted. A copious and viscid growth is obtained on an agar slope. Both acid and gas are produced in the litmus carbohydrate media.

The influenza bacillus The bacilli in the earlier stages of the disease may be found in very large numbers in the sputum. They occur in considerable sized clumps outside the cells, and a minority of them are intracellular. The minute "dew drop" colonies may be obtained in plate cultures on blood agar. The colonies grow somewhat slowly, and the organisms, unless particularly abundant, are apt to be overgrown by other bacteria.

Gram stained film preparations of the sputum counter stained with carbol fuchsin are useful as a means of diagnosis. The minute red bacilli are sufficiently characteristic.

Streptococci These organisms are very constantly met with in the sputum, but are usually not hæmolytic. Perhaps the commonest bacterium met with in all samples of sputum is a non hæmolytic streptococcus of the viridans type. *S. viridans* and non hæmolytic streptococci probably play a part in a variety of pulmonary infections, but mainly as secondary invaders of the lung. They are organisms often predominant in the contents of a bronchiectatic cavity.

Staphylococci These organisms are frequently found in the sputum, and may be responsible for a percentage of bronchitis cases. *S. aureus* and *S. citreus* are much less commonly met with than the white staphylococcus, which is found in practically all sputa and has no diagnostic significance.

Diphtheria bacillus This bacillus is rarely found in the sputum, but in cases of laryngeal diphtheria with a membrane spreading downwards portions of the membrane may be occasionally coughed up. Membrane is commonly expelled from a tracheotomy wound, and there is little difficulty in cultivating the bacillus from it.

Incent's organisms Both the spirilla and the fusiform bacilli may on occasion appear in considerable numbers in the sputum and are believed by some authorities to be the infective causes of certain chronic pulmonary affections resembling tuberculosis in their clinical course

Actinomyces Actinomycosis of the lung is an uncommon condition but the organisms may obtain primary lodgment in the lung or may be carried there from a distant focus. The characteristic granules can be found in the sputum or in the pus from an empyema and should be examined in the ordinary way. The beaded Gram positive streptothrix is sufficiently characteristic but clubs are practically never met with. Other streptothrices are occasionally found and some may be both Gram positive and acid fast thus resembling the long and beaded forms of the tubercle bacillus. The diagnosis of a streptothrix infection from the sputum must be made with care since streptothrix like organisms may be present in the mouth as a contamination from the food and it is advisable that the mouth should be thoroughly cleansed before the sample of sputum is obtained. The actinomyces organism occurs in tufts (see Plate VIII) of beaded filaments and should not be confounded with the long thin beaded bacilli often present in sputum nor with the fine fibrinous filaments seen in all film preparations from sputum or mucopus.

The plague bacillus In the pneumonic forms of plague the causative organism is to be met with in large numbers in the sputum. The bacilli are present also in the septicæmic form of plague and in the bubonic form but only in the latter condition when there is a pyæmia with metastatic abscesses in the lung. The diagnosis in a plague district may reasonably be made from the appearance of the bacilli in film preparations. The bacilli occur in the sputum in pairs, clumps and short chains. The bipolar staining is best seen if the films are fixed in absolute alcohol before staining. Cultures of the bacillus should be obtained if possible but the organism is often outgrown by the other bacteria of the sputum.

Lung puncture Puncture of the lung through the chest wall as a means of diagnosis has already been referred to but since it is a proceeding not devoid of risk is rarely justifiable. The operation is not infrequently performed accidentally in the expectation of finding fluid in the pleural cavity and the small quantity of blood stained fluid removed from the lung is often

sufficient for film preparations and for cultural processes. The bacteriological examination of fluid removed in this way is more satisfactory than similar investigations of the sputum.

Pleural fluids The nature and mode of examination of these fluids has been described in the section on "Puncture Fluids."

BASAL METABOLISM

The basal metabolic rate is the amount of energy used in a given time when the subject is at complete rest. Standards have been worked out allowing for the height, weight and age, from which the basal metabolic rates of normal subjects deviate little.

The most important deviations from these standards are found in diseases of the thyroid, in hypothyroidism the rate may fall to 60 per cent. of normal, and with hyperthyroidism rise to 170 per cent. of normal. This rise in the metabolic rate runs fairly closely parallel with the increase in pulse rate.

Since the amount of energy given out when a litre of oxygen is used in oxidation is approximately the same whatever the food burnt, the metabolic rate can be calculated from the rate at which oxygen is used up, simple apparatus based on the rate of oxygen consumption are on the market. A more satisfactory, but less simple, method is to collect the patient's expired air in a Douglas bag, measure the amount expired in a given time and the oxygen and CO_2 content. Knowing the oxygen content of the external air, the amount used up can be calculated. The respiratory quotient can also be calculated, and more information about the food burnt obtained thereby.

When these methods are attempted on a patient for the first time considerable alarm is caused unless the patient has seen others similarly investigated, this alarm produces a rise in the metabolic rate. For this reason results are misleading unless more numerous estimations than are possible to most clinical pathologists are made. Although the results are theoretically interesting they show little more than can be learnt by ordinary clinical examination.

It should not be forgotten that the standards are not applicable to persons with much fat or oedema, as they carry a large amount of dead weight.

SECTION VIII.—HISTOLOGY

CHAPTER XXV

THE EXAMINATION OF SECTIONS—THE INFLAMMATIONS—THE DEGENERATIONS

THE EXAMINATION OF SECTIONS

THE histological examination of tissues removed during life is among the most important of the methods of clinical pathology, and from the point of view of diagnosis probably the most difficult. There is no other laboratory procedure which requires more experience for the interpretation of what one sees and the necessary experience cannot be gained by reading but comes from repeated examination of many types of the same pathological change.

An acquaintance with the normal histology of the tissues is essential and the student is advised not only to examine, whenever possible type specimens of pathological conditions, but also to renew his acquaintance with sections of the normal human tissues.

Methods of examining sections. The most convenient microscope for the majority of histological examinations is one without a mechanical stage. The most useful powers are a Zeiss 2 eye piece with a 1 inch and $\frac{1}{6}$ inch objective. For the occasional more searching study of the structure of particular cells and small bodies a $\frac{1}{12}$ inch objective should occupy the third place on the triple nose piece. An ordinary hand magnifying glass is often of considerable assistance.

The sections should first be examined with the naked eye, and points of considerable importance can frequently be made out. The naked eye inspection can be amplified by holding the slide against the light and examining it further with the hand glass. The distinction between the normal tissue and the abnormal is often obvious, and the general aspect of the section is of great value when considering the microscopic appearance of a series of "fields." The entire area of the

section is then carefully gone over with the 1 inch objective. Under this power the relation of the normal to the abnormal tissues is definitely made out, the grosser structures are recognised, and to a less extent the relation of the cellular elements to the connective tissue is observed.

The $\frac{1}{2}$ inch objective is used last and most sparingly. The portions of the section to be examined more particularly will have been indicated by the previous inspection. The points to be considered are the nature of the cells, their size, shape, and staining reaction, and the character of their nuclei. The relation of the cells to each other and to the connective tissue network is further investigated. The observer who pays too much attention to the higher magnification and too little to the general examination under the low powers is in danger of 'not seeing the wood for the trees'. Further the naked-eye appearance of the tissues removed must be considered.

The following brief description of the various processes of morbid histology can obviously not be expected to give a detailed account of the various changes which may occur.

THE INFLAMMATIONS

The inflammations may be divided into acute and chronic. The chronic may be subdivided into the chronic purulent or pyogenic (pus forming) and the granulomata. Among the granulomata is a special group, the specific granulomata, comprising tuberculosis, syphilis, rheumatic fever, Hodgkin's disease and leprosy.

Acute inflammation. This occurs usually as the result of irritation by the toxins of the pyogenic organisms such as staphylococci, streptococci, pneumococci or gonococci and less commonly by mechanical, chemical or other irritants, *e.g.* formalin.

In its early stages acute inflammation is characterised by an exudative type of reaction, there is exudation of blood plasma, formation of fibrin, migration of leucocytes from the blood vessels and the appearance of other round cells. In its later or granulating stage there is a formative type of reaction, when granulation tissue develops, composed of spindle cells, collagen fibrils (white connective tissue) and new capillaries. Besides all this reaction in the interstitial tissue, which is the inflammation proper, the poison or toxin producing the

inflammation may effect changes in other cells which may be present such as epithelial or parenchymatous cells *e.g.* the cells of the liver or kidneys

The changes in epithelial and parenchymatous cells are either proliferative or degenerative. Epithelial cells such as those of the epidermis tongue larynx renal pelvis etc. may become damaged and cast off when involved in an acute inflammation and proliferation of the remaining cells takes place to repair the defect. the epithelium when not damaged may become thickened by proliferation. The columnar epithelial cells of mucous glands and ducts distend themselves with mucus and are desquamated this process being the main characteristic of catarrhal inflammation. The epithelial cells lining the alveoli of the lung proliferate and act as phagocytes. Parenchymatous cells of the kidney and liver undergo various degenerative changes ranging from cloudy swelling to necrosis

Changes in vessels Although the alterations in rate of flow of blood can obviously only be seen in the living animal yet in sections dilatation of all the thinnest vessels of the part is often conspicuous. Relative increase in the number of leucocytes in the blood vessels is also seen. In the later stages of acute inflammation the small vessels project solid buds of endothelial cells which become hollowed out to form new capillaries in the granulation tissue. The exudation of plasma (*inflammatory œdema*) can often be appreciated in a section the spindle cells fibrils and other tissue elements being separated from one another by homogeneous hyaline coagulated albumin. The thickening thus produced often simulates fibrosis but this point can be settled by a Van Gieson stain in which the fluid is yellow fibrous tissue being red. This inflammatory effusion is seen in large cavities such as the pleura but in microscopic sections it may also be seen in small cavities such as acini or pulmonary alveoli. The fluid frequently clots owing to the action of fibrinokinase given off from dead cells and the *fibrin* thus thrown down is a common characteristic of early acute inflammation and is most conspicuous on serous surfaces such as the peritoneal surface of the appendix and in any small cavity within the tissue. The fibrin appears as irregularly disposed thick and thin filaments stained red with eosin and yellow with Van Gieson's stain and blue with the Weigert Gram method

Red blood corpuscles escape passively from the vessels and are found free in the tissue, though this is not a salient feature of the ordinary acute inflammation. It occurs to a great degree however in certain inflammations, such as anthrax, and quite frequently in acute inflammation of the gall bladder, the inflammation is then described as "hæmorrhagic." Surgical procedures, such as clamping an appendix during its removal, can induce hæmorrhage into a tissue.

Round celled infiltration The active migration of leucocytes from the blood vessels and the formation of other types of round cells are among the most obvious and important changes in inflammation. These various round cells are found between the elements of the interstitial tissue and in the lumen of such ducts and acini as may be present, or are seen entangled between the filaments of the fibrin on the surface of an inflamed part.

The *polymorphonuclear neutrophil leucocyte* is the predominant migrating round cell in early acute inflammation. Its presence in large numbers means an intense inflammation. In a paraffin section stained with hæmatoxylin and eosin it is easily recognised under the $\frac{1}{6}$ inch objective. It appears as a small round cell with relatively faintly eosinophil cytoplasm and a deeply hæmatoxyphil nucleus composed of two, three or more lobes. In severe acute inflammation an area of body tissue may be destroyed by ferments set free by these leucocytes and the cavity thus formed is filled with pus. The chief constituent of pus as seen in sections is the neutrophil polymorphonuclear leucocyte, for the most part degenerate and densely packed in vast numbers, other recognisable structures in pus are micro-organisms and fragments of dead tissue cells and fibres. Digestion and replacement of a circumscribed area of tissue by pus cells constitute an *abscess*. Bacteria may be seen in the neutrophil leucocytes.

The *eosinophil leucocyte* is not so constantly found, but when present is a very conspicuous feature in a hæmatoxylin and eosin section, owing to its coarsely granular, bright red cytoplasm, its nucleus in an inflammatory infiltration is most frequently bilobed, but is sometimes without lobation. Eosinophils are numerous in certain special inflammations, such as those due to animal parasites, but they may also abound in the infiltration in the later stages of any common pyogenic infection.

Small lymphocytes are found in an acutely inflamed area, but are much more numerous in chronic inflammations. They migrate from the blood and lymph vessels, but most authorities are agreed that they are also formed by proliferation *in situ* in the inflamed area. They are easily recognised in sections as the smallest round cell present, having very little basophil cytoplasm and a deeply stained round nucleus.

The *plasma cell* is usually present in the later stages of acute and in chronic inflammation. Clumps of densely packed plasma cells are often found in purulent inflammations of some standing. They apparently arise locally from lymphocytes in the inflamed tissue and are to be regarded as altered lymphocytes. They are several times as large as a small lymphocyte. They are easily recognised in a hæmatoxylin and eosin preparation by their egg like shape, abundant basophil cytoplasm and eccentric nucleus, the nucleus has been likened to the face of a clock, having nodes of chromatin on the inner side of the nuclear membrane. A cell should not be called a plasma cell unless the nucleus has this structure, cells which have the above characteristics without the clock face nucleus are called *plasmacytoid* cells. The plasma cell does not appear to be a phagocyte, and its function in inflammation is unknown. It is a common inhabitant of the mucosa of the gastro intestinal tract under normal conditions, as indeed are lymphocytes and eosinophil leucocytes, and this must be kept in mind when considering the diagnosis of inflammation in this area. the normal presence of these cells is a real stumbling block in the diagnosis of the milder degrees of appendicitis.

Characteristic examples of all the above round cells are easily recognised in sections, but there are also numerous large round cells found in the later stages of acute and in chronic inflammations. These have various origins, and present such slight differences from one another in structure *that it will suffice to group them together under the name of large mononuclear cells*. They are generally somewhat larger than plasma cells, have a large round or indented, lightly stained nucleus with distinct chromatin net and nucleoli and a moderately abundant faintly basophil cytoplasm. They are active phagocytes, and in addition to bacteria are often seen to contain fragmented or complete neutrophil leucocytes, red blood corpuscles and blood pigment, as well as other

pigments and *debris*. These are Metchnikoff's *macrophages*. They are also called *large wandering phagocytic cells*. Mallory would name nearly all of them *endothelial leucocytes*. They probably have many origins and belong to what is now called the "reticulo-endothelial system." This "system" comprises various cells in different parts of the body, which were first grouped by Ribbert in 1904 because during *intravital* staining he found they took up carmine. It is a "system" founded on function and not on similarity of origin. These cells are the reticulum cells of the spleen and lymphatic glands, the endothelial cells of the venous sinuses of the spleen and the lymph sinuses of the lymphatic glands, Kupffer cells (large capillary endothelial cells) in the liver, endothelial cells of the marrow, and "histiocytes" or cells found in the dermis and in the connective tissue of most organs. All these cells are concerned with the destruction of red blood corpuscles in normal and abnormal conditions, and they also give rise to the large mononuclear phagocytes of inflammation, but some of the latter also arise, according to Mallory, by the division and migration of the endothelial cells of small vessels. The large mononuclear leucocyte or hyaline cell of the blood is thought to belong to this system.

The *fibroblasts*, as the spindle cells of fibrous tissue are called, proliferate in the later stages of acute inflammation and in the stage of repair. The newly formed fibroblasts may be much larger and stouter than the cells from which they took origin. They vary from a spindle to a round or stellate shape. The majority have oval or spindle shaped, lightly stained nuclei, and moderately abundant lightly stained cytoplasm, which is extended at the various angles of the cell into long tapering processes. They secrete outside themselves collagen fibres (white connective tissue fibres). The newly formed collagen fibres are stained yellow by Van Gieson's stain, but soon they are stained pink, and finally, as the formation becomes dense in late inflammation or repair they may take a deep red tint. In the inflamed area the capillary endothelial cells divide to form *new capillaries* which connect up with one another to provide a fresh circulatory system.

In the late stage of inflammation the round cells gradually disappear, the collagen fibres increase, become more densely packed and arranged parallel in bundles, while the fibroblasts

become fewer and revert to their original narrow spindle form and the majority of the new capillaries close and vanish. The inflamed tissue thus changes into a relatively anæmic scar. Elastic fibres gradually develop in the scar.

In inflammation of certain tissues other cells may play a part: the pulmonary alveolar epithelial cells and the serous endothelial cells of the pleura, peritoneum and pericardium are examples of actively phagocytic large round wandering cells.

Generally speaking therefore the following are the features to be seen in a section of tissue in a state of well



FIG. 28.—Granulation Tissue. Showing Polymorphonuclear and Plasma Cells and Fibroblasts. Drawn under $\frac{1}{2}$ inch Objective.

established acute inflammation: newly formed young fibroblasts arranged irregularly; delicate new collagen fibres; an abnormal number of capillaries; faintly eosinophilous hyaline substance which is exuded plasma separating the tissue elements (inflammatory oedema); filaments of fibrin; round cells of various types and in varying proportions, viz., neutrophil and eosinophil leucocytes, lymphocytes, plasma cells and large mononuclear cells. An inflammatory tissue composed of these elements is called *'granulation tissue'*.

The organisms causing the inflammation may often be found in abundance in sections stained with Gram's or other methods, and indeed they are often recognised easily in sections

stained with hæmatoxylin, but success in this matter may depend largely on the patience of the searcher

In less intense acute inflammation or subacute inflammation, the infiltration is relatively sparse, and neutrophil leucocytes are scanty or absent

Chronic pyogenic inflammation results when an infection by pyogenic organisms is checked by the reaction of the tissues, but still persists, it is frequently associated with the presence of infected foreign bodies, concretions or portions of necrosed bone. Conspicuous infiltration by neutrophil leucocytes, with or without the formation of abscesses, is combined with abundant fibroblastic proliferation, fibrosis and infiltration with lymphocytes, plasma cells, eosinophil leucocytes and large mononuclear cells

"Fat granule cells" or "foamy" cells, though found in other conditions, are specially characteristic of chronic purulent inflammation. These are phagocytes, which load themselves with the fatty and lipid substances set free during inflammatory destruction of tissue. They are several times the size of a plasma cell and in a paraffin section, when the ingested fats have been dissolved in the process of embedding, the cytoplasm has an extremely rarefied, vacuolated or "foamy" appearance, the nucleus being relatively small and round. These cells often form groups large enough to be seen with the unaided eye as yellow specks and streaks

Human *actinomycosis* should be classed among the chronic purulent inflammations. It is not a true granuloma, as it is always associated with the formation of pus and some of its lesions are simply acute abscesses. The more chronic actinomycotic lesion is a mass of fibrous granulation tissue permeated with abscesses, it has no special histological features, but fortunately the causative organism is frequently conspicuous in the abscesses

A granuloma is an inflammation in which proliferation of fibroblasts and other cells of the fixed tissues is such a salient feature that "tumours" of granulation tissue are liable to be formed. Any chronic inflammation, whether purulent or not, can form a tumour like mass of granulation tissue and may therefore be called a granuloma, but it is convenient to confine the term granuloma to histological reactions which are characterised by conspicuous proliferation of the fixed tissues (formative reaction) and by little or no exudative phenomena

According to this histological definition the granulomata are non purulent

A granuloma can be produced by various forms of irritant, *e.g.*, inhalation of dusts in pneumokoniosis, but the most typical examples are seen among the diseases known as the specific granulomata, which are so named because each displays more or less specific characters

Tuberculosis The most characteristic tuberculous lesion is the granulomatous tubercle. The centre of a typical granulomatous miliary tubercle is occupied by one or more multinucleate giant cells and several rounded or polygonal cells, which from the abundance of their cytoplasm are known as epithelioid cells. Round these there is usually a zone of more spindle shaped cells. In the periphery of the tubercle is a zone of infiltration with lymphocytes. The giant cells usually have ill defined borders, central pallor and vacuolation of the cell body, and peripherally placed pale oval nuclei. More spindle cells are seen as the process continues, and between these some collagenous fibres are developed. Characteristics of the epithelioid and spindle cells are the poor definition of the cell bodies and the swollen and lightly stained appearances of the nuclei. Plasma cells are rarely found in the infiltration round a granulomatous tubercle and eosinophil leucocytes almost never occur. In the caseous tubercle the centre is occupied by an area of necrosis, called caseation because of its macroscopic resemblance to cheese. Round this is a narrow zone of granulation tissue similar to that which forms the granulomatous tubercle. The caseous area consists of granular *débris* stained by eosin in which a few small fragments of nuclei are deeply stained by hæmatoxylin. In Van Gieson preparations collagen fibres and ghosts of cells can seldom be recognised, because these are rapidly disintegrated. Elastic fibres are also rapidly destroyed in tuberculous caseation.

Liquefaction or autolysis may result in the formation of a creamy fluid from the caseation—so called “tuberculous pus”—but this contains only a few neutrophil leucocytes, whereas the true pus of the pyogenic inflammations is packed with them.

Tuberculous inflammation is usually, but not invariably, granulomatous. In acute tuberculosis, particularly of the bronchioles and alveoli of the lung the inflammation may be

entirely exudative. An exudation of plasma from which fibrin is precipitated is associated with infiltration by neutrophil leucocytes and proliferation and desquamation of alveolar epithelial cells. The process can only be distinguished from that due to pyogenic organisms for instance the pneumo-

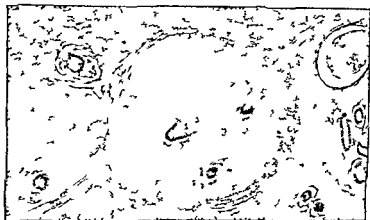


FIG. 29.—Tuberculosis of Kidney. Drawn under $\frac{1}{4}$ inch Object ve



FIG. 30.—High Power Drawing of a giant Cell
in Fig. 28

coccus by the presence of tubercle bacilli and the tendency to caseate. In tuberculous inflammation of the serous sacs exudation into the sac of plasma with the precipitation of fibrin is usually combined with granulomatous inflammation of the wall of the sac.

Phlebitis and arteritis with great thickening of the intima

by granulation tissue (endophlebitis and endarteritis) are frequently found in tuberculous lesions, but are not so constant as in gummatous syphilitic reactions

The diagnosis of a tuberculous lesion is not complete without the identification of tubercle bacilli. Sections of tuberculous lesions suitably stained are occasionally found to be swarming with tubercle bacilli, but as a rule bacilli are scanty, and a negative result of a search for them has therefore a value in direct proportion to the extent of the search

Syphilis The histological diagnosis of *congenital syphilis* arises more in post mortem work than in the clinical laboratory, so it will be dealt with briefly. In infants the liver is the seat of a very widespread pericellular diffuse fibrosis. Osteochondritis is found at the growing parts of long bones. The liver and spleen are infiltrated with myelocytes, myeloblasts and other primitive blood cells indicating a persistence of the foetal blood formation in these organs. Vast numbers of spirochaetes are found in the suprarenals and in the liver, in other tissues they are usually less numerous. Fibrous pneumonia (‘grey pneumonia’) is a rare lesion. In older children congenital syphilitic lesions are similar in their histology to those of acquired syphilis but tend to be bilateral.

Acquired syphilis The *primary chancre* has no very special histological features. It is a localised area of spindle celled proliferation with formation of a little collagen fibre swelling and proliferation of the intimal and adventitial cells of the small vessels and diffuse infiltration by plasma cells and other round cells. Neutrophil leucocytes are only found close to the ulcer that is usually present.

The *secondary lesions* (cutaneous papules, mucous patches and condylomata) show an inflammatory infiltration similar to that of the chancre, and occasionally groups of epithelioid and small multinucleate cells. There is also a variable degree of proliferation of the overlying epithelial cells. Spirochaetes tend to lie in the epithelium. The enlarged glands are usually crowded with plasma cells.

Tertiary lesions are occasionally sent to the pathologist for examination. Gummata of the skin, muscles, tongue or testicle are sometimes mistaken for neoplasms and so removed.

A typical *gumma* has a relatively small area of necrosis or gummatous caseation in which, in contrast to tuberculous caseation, many collagen fibrils and ‘ghosts’ of cells without

nuclei are distinguishable, elastic fibres also persist for a longer period than in tuberculosis. Around this is a relatively broad zone of very fibrous granulation tissue consisting of spindle cells, numerous collagen fibres and an abundant infiltration with round cells, especially plasma cells, but also eosinophil leucocytes, lymphocytes and large mononuclear cells. Strands of fibrosis extend widely into the surrounding tissue. The arteries and veins in and near a gumma show endophlebitis and endarteritis having their intima greatly thickened by spindle and round celled granulation tissue whilst their adventitia and to a less extent their media are affected in the same way. Giant cells not infrequently appear near the border of the necrosis. They may be absolutely identical with the typical tuberculous giant cell, but usually are smaller and more sharply defined, they are frequently absent. The zone of granulation tissue surrounding the area of gummatous necrosis is typically very wide, but occasionally narrow.

A definite diagnosis can be made when one is dealing with a typical gumma, but it is generally admitted that in many cases it is impossible to differentiate on histological grounds between gumma and tuberculosis. A useful practical point to remember is that small granulomatous tubercles of typical structure or more commonly small nodules composed of swollen ill defined epithelioid cells, are usually found in the periphery of a large tuberculous area. Further, gummata are exceedingly rare in the lymphatic glands, brain, kidney and spleen. Spirochaetes unfortunately have rarely if ever, been found in gummata.

Non gummatous syphilitic inflammations, which are typical of the late or parasyphilitic stage are still more difficult to diagnose with the microscope. They are lesions of a less intensity than gummata. They are characterised by an infiltration with lymphocytes, plasma cells and a few eosinophil leucocytes and a slowly progressive fibrosis. Such lesions are found in the testicle, myocardium, aorta, periosteum, etc. and in the meningo-encephalitis of general paralysis. Endarteritis is typically absent.

In difficult cases it is obviously of great importance to sum up all the evidence—histological, serological and clinical.

Around foreign bodies, such as sutures, giant cells and epithelioid cells may be found. The foreign body giant cells show more multiplicity of shapes than the tuberculous or

syphilitic giant cells and their nuclei tend to be scattered through the cell. The foreign bodies are usually conspicuous sutures are pale brightly refractive objects in a section and the giant cells tend to be curved so as to envelop them.

Hodgkin's disease There can be no doubt that this is a granuloma. The harder glands give the more characteristic changes. The infiltration quickly replaces the whole structure of the glands so that few if any germ centres and lymphatic sinuses can be recognised. The reticulum cells of the glands



FIG. 31.—Lymphatic Gland in Hodgkin's Disease. Drawn under $\frac{1}{4}$ inch Objective.

proliferate and give rise to large round, oval and spindle cells from these are formed giant cells the most typical of which show the greater part of the cell occupied by many superimposed pale nuclei with very large nucleoli. Owing to protoplasmic shrinkage in the fixative the giant cells frequently seem to lie in spaces. Other forms of giant cells are frequent such as those with a deeply stained tortuous mass of chromatin suggesting the nucleus of a megakaryocyte. The proliferated reticulum cells of various sizes and shapes mixed up with collagen fibrils, lymphocytes, a few plasma cells, sometimes numerous eosinophil leucocytes and scattered giant cells of

different sizes make up a very characteristic picture. In later stages collagen fibres are increased whilst the free cells become scantier until ultimately dense hyaline fibrous ribbons may be predominant. Areas of necrosis very similar to tuberculous caseation are not uncommon. The typical picture described is not invariably shown. Some lesions may be composed of large round cells of uniform type resembling endothelial cells. Differential diagnosis from lymphosarcoma is then impossible.

Rheumatic granuloma hardly ever comes into the practice of the clinical laboratory. It is seen in rheumatic endocarditis, myocarditis, etc. The granulomata are composed of spindle and triangular fibroblasts, collagen fibrils, comparatively small multinucleate giant cells rather of the Hodgkin type and an infiltration with lymphocytes and other round cells and they usually show central fibrinoid degeneration. Ultimately the inflamed area becomes densely fibrotic and the fibrotic tissue is frequently calcified.

Leprosy. In an active nodule from the skin there is dense infiltration of the dermis with large epithelioid cells and small round cells such as lymphocytes. The diagnosis is usually easily made by the finding of large numbers of Hansen's acid fast bacilli grouped inside the epithelioid cells. They are to be stained by the same method as that given for tubercle bacilli in sections (p. 471) but 12 per cent. acid must be used for decolorising in place of 25 per cent. In old leprosy lesions there may be simply a dense fibrosis of the dermis.

Mycosis fungoides is a chronic granulomatous inflammation. The lesions are characterised by an infiltration with large and small lymphocytes, large mononuclear leucocytes, plasma cells and eosinophil and neutrophil leucocytes associated with a proliferation of fibroblasts or of reticulum cells, these processes leading at first to the formation of massive cellular nodules and later of scars. Eosinophil leucocytes are usually abundant and conspicuous, the fibroblasts in the early stages are spheroidal but later become spindle shaped, there are occasionally large giant cells with numerous small oval nuclei that are usually disposed peripherally. The granulomatous nodules occur for the most part in the skin but similar nodules may be present within the body, for instance in the mucosa of the tongue, in the myocardium and in the liver whilst in the lymphatic glands and spleen there is a general inflammatory

infiltration of similar cytology with or without focal areas of sclerosis

Molluscum contagiosum occurs in the form of small umbilicated nodules in the skin and is definitely contagious. It may be found in any part of the skin and may be inoculated from one part to another. The tumours may develop though rarely on the penis and be mistaken for chancres.

Sections through a nodule have a very characteristic appearance. Under the low power the nodule is seen to be composed of a number of club shaped invaginations of the epidermis and of isolated transverse sections of the deeper portions of such invaginations. Each invagination is bounded externally by a basal layer followed internally by a narrow zone of prickle

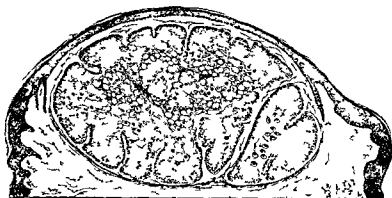


FIG. 3.—*Molluscum Contagiosum*. Drawn under $\frac{1}{2}$ inch Objective

cells which are usually rounded and slightly swollen. Internal to this the cells become grossly swollen and rounded; the cytoplasm is occupied by round eosinophil bodies in the meshes of a delicate net of hæmatoxyphil threads, and the nucleus is flattened upon the surface. In the stratum lucidum and corneum the swollen cells become hyaline deeply eosinophil spheres with a nucleus upon the surface.

THE DEGENERATIONS

The degenerations are more often studied in post mortem work. Some of them are occasionally seen in tissues removed during life, so a brief account of the commoner will be given here.

Albuminous degeneration (cloudy swelling) This is an

extremely common condition, occurring usually in chemical or bacterial intoxication. It is found in the epithelial cells of the cortical tubules of the kidney, the hepatic parenchyma cells and the myocardial fibres. In a fresh, unfixed, unstained section, mounted in saline, the cytoplasm is full of obvious granules which obscure the nucleus and which disappear when dilute acid or alkali is introduced under the cover slip. In paraffin sections the cells are still obviously granular and very swollen, in the kidney they partly obliterate the lumen of the tubules. The nuclei show the degenerative change known as *chromatolysis*, in which the nucleus is stained very faintly and eventually may disappear. Collection of the chromatin of the nucleus into a deeply stained clump may also be seen and is known as *pyknosis*. If the cells imbibe fluid and swell still more, and vacuoles appear in the cytoplasm, the condition is called *hydropic or vacuolar degeneration*.

Mucous or mucoid degeneration is found in fibro-cartilage and is practically the only change ever to be found in the commonly removed semilunar cartilage. A mucous or mucoid change occurs in chondromata, sarcomata and carcinomata. In the tissue affected there is a development of gelatinous material which in paraffin sections stained with hæmatoxylin and eosin is composed of very fine threads and granules stained a faint blue, they are stained bright red with muci carmine stain.

Hyaline degeneration The term hyaline does not denote any specific change, but is used to describe any change under which a tissue assumes a homogeneous glassy appearance like cartilage matrix. The commonest is *hyaline fibrous degeneration*, where the fibrils of fibrous tissue swell up and fuse together and are stained deep red with Van Gieson's stain. It is very common in arterial degenerations, scar tissue and fibrous neoplasms. In other hyaline degenerations the material is stained yellow with Van Gieson, this is sometimes seen in small arteries in the kidney. Little is known concerning the chemical nature of these changes.

Hyaline droplet degeneration occurs in the tubular epithelium of the kidney in certain forms of Bright's disease. Round droplets of various sizes, often as large as a red corpuscle, and staining brightly with eosin, appear in the cytoplasm. The droplets are Gram positive.

Amyloid or lardaceous degeneration is an example of a "hyaline" change the nature of which is known. It occurs

especially whenever there is prolonged suppuration as in chronic bronchiectasis or secondary pyogenic infection of tuberculous or syphilitic lesions and more rarely in chronic inflammation such as the granulomata without suppuration. There is a deposit of the amyloid material (chondroitin sulphuric acid combined with a protein) in connective tissue fibrils especially those of the walls of the small arterioles and arteries. The fibrils swell and eventually whole areas of tissue as in the liver may be replaced by the amyloid material the tissue cells undergoing fatty degeneration and necrosis from deficient blood supply.

In paraffin sections stained with 2 per cent gentian or methyl violet and washed with 2 per cent acetic acid the amyloid material is stained pink.

Fatty changes *Fatty infiltration* is an increase of fat in cells which normally store fat in a visible form *i.e.* adipose connective tissue cells and the hepatic parenchyma cells. The change is simply an adiposity—an excess of food material is brought to the cells and is not metabolised but is stored. The fat tends to form a single large globule distending the cell. The nuclei show no degenerative changes. The causes are those of obesity—excess of nutriment and disturbances in the endocrine glands.

In *fatty degeneration* fat appears in cells which do not normally contain it in a visible form *e.g.* the renal epithelium and cardiac muscle cells but it also affects the hepatic parenchyma cells. The fat is usually in multiple small droplets in the cells. The nuclei show degenerative changes—chromatolysis and pyknosis. Following fatty degeneration cells frequently go on to necrosis when the nuclei may show karyorrhexis or breaking up of the nucleus into deeply stained fragments. Fatty degeneration is caused by chemical and bacterial poisons or deprivation of nutriment. It is invariably preceded by a stage of cloudy swelling. In fatty degeneration the fat is partly brought to the cells from elsewhere and is partly derived from the protoplasm of the cells. The fatty substances are dissolved out by alcohol and chloroform in the process of preparing paraffin sections when large vacuoles are left in the cells the condition can be identified but this is not so easy in the case of small droplets. In order to retain the fat in the tissues frozen sections are necessary and they may be stained with Scharlach R or Sudan III.

In fatty degeneration the fatty substances in the cells are neutral fats and fatty acids. *Lipoidal degeneration* also occurs, and the lipoids that appear in the cells are mainly cholesterol esters. Fatty and lipoidal degenerations frequently occur together. Fats and lipoids are stained almost the same tint with Scharlach R and Sudan III, but when unstained frozen sections are examined under the polarising microscope, the lipoids are seen to be doubly refractile and the neutral fats are not. Lipoids are found in various forms of Bright's disease, but also occur in atheroma and many other conditions, and are abundant in normal suprarenal cortex.

Calcareous impregnation Calcium is deposited in the tissues in many conditions, chiefly in areas of necrosis, especially caseous necrosis, and in arterial degeneration. In order to obtain sections it is usually necessary to decalcify the tissue partially. Calcium is very conspicuous in sections as the granules are stained very deeply with hæmatoxylin.

Pigmentation Pigments that are formed inside the body (*endogenous*) are chiefly melanins, lipochromes and derivatives of hæmoglobin. The latter are hæmosiderin, bile pigment (bilirubin, etc.), and hæmofuscin. *Hæmosiderin* is extremely commonly seen in sections, appearing as fine brownish granules. It contains the iron of the hæmoglobin, and when a section is appropriately treated (p. 472) the pigment is stained greenish-blue (Perl's test or Prussian blue reaction). It is found in phagocytes after hæmorrhage into tissues, and in hepatic, renal and other cells in pernicious and aplastic anæmias, also in hepatic, pancreatic, lymphadenoid and other cells in hæmochromatosis, a condition characterised by abnormal retention in the body of hæmosiderin and hæmofuscin. In jaundice bile pigment is seen in hepatic, renal and other cells, as a green or a yellowish pigment, which gives Gmelin's reaction.

Melanin appears as brown or brownish black pigment, and in a section closely resembles hæmosiderin, but can be distinguished by the fact that it does not give Perl's test. It occurs in the skin in freckles, in Addison's disease and in many skin conditions, including melanotic neoplasms.

Lipochromes are the fatty pigments. Yellowish brown lipochrome pigment is found in the protoplasm of cardiac muscle cells at either pole of the nucleus in brown atrophy of the heart, and in hepatic cells in brown atrophy of the liver.

That this pigment is a lipochrome can be demonstrated by staining with Scharlach R or Sudan III

The chief pigments that are taken into the body from without (*exogenous*) get in by the airway and give rise to a large group of conditions known as pneumokoniosis. The commonest are *anthracosis* (inhalation of soot or coal dust) and *silicosis* (inhalation of dusts containing silica). These particles can be recognised in the lung and bronchial glands.

breast and in certain cysts of the ovary. In the breast the core is usually composed of a great number of repeatedly branched processes and these are covered by a double row of cubical epithelial cells similar to those which line the normal ducts. In malignant examples the covering consists of several cells and these usually vary in size and shape. Owing to the branching of the core in both examples and consequent tangential section it is frequently very difficult to decide whether the covering is composed of a double row of cells arranged typically or of several rows of cells arranged irregularly. Inasmuch as the benign examples are liable to become malignant it is expedient to regard the condition as malignant when there is difficulty in deciding this point.

Other adenomata arise within the substance of glands. They are composed of tubules or alveoli lined with epithelium similar to that which lines the normal ducts or alveoli. The tubules and alveoli are embedded in a stroma of vascularised fibrous tissue. Discrimination is sometimes difficult between adenomata and diffuse overgrowth of a gland as in the thyroid. Enlargement of the thyroid or goitre is apart from malignant neoplasms divided into diffuse and nodular. In diffuse goitre there is uniform enlargement of the thyroid. It is subdivided into colloidal and a colloidal. In diffuse colloidal goitre the thyroid is composed of acini of varying size full of typical colloid and lined with small cubical or flattened cells with a well defined inner border. Diffuse a colloidal goitre is the form found in Graves disease. Here the acini contain no typical colloid or relatively little. the fluid in the acini tends to be lightly stained and thin looking. the majority of the cells are large even columnar with ill defined inner borders and large nuclei. occasional cells may have large hyperchromatic nuclei or more than one nucleus. small papillae may project into acini or desquamated cells lie in the acini. some acini have no lumen being solid masses of cells. lymphoid nodules lie in the connective tissue between the acini. After a patient has had iodine treatment many of the acini may contain colloid. In nodular goitre the enlargement of the thyroid is due to the presence of adenomata but the intervening thyroid tissue may itself be in a state of hyperplasia and in some cases the adenomata are hardly true neoplasms being merely local exaggerations of the changes in the rest of the gland. Adenomata are colloidal or a-colloidal.

Colloidal adenomata may be single but are usually multiple. Their structure is that of diffuse colloidal goitre. They may be separated by a fibrous capsule from the surrounding thyroid tissue whose acini are flattened from compression. A colloidal adenomata are of various forms. Many of them are not entirely a colloidal but they are mainly composed of solid strands or acini of small or large cells. One variety is important in that it is liable to become malignant. It is composed of columnar cells lining long branching tubules which may contain papillae and may be cystic. Adenomata of the thyroid are clinically divided into toxic and non toxic. Examination of the histological structure does not enable one to state with accuracy whether an adenoma is toxic or not but the majority of the toxic adenomata are of the a colloidal variety. In the thyroid in all forms of goitre but especially in the nodular form the following secondary changes are common: cysts from distension and fusion of acini; fibrosis which may be extensive and hyaline; calcification; recent or old hæmorrhage; necrosed tissue containing cholesterol crystals.

In some adenomata the connective tissue is in greater amount than is usual in the normal gland and then the term *fibro adenoma* is used. This is common in the breast. In mammary adenoma the stroma frequently grows to such an extent as to flatten the tubules of the adenoma and convert them into branching slits. The stroma as it were grows into the tubules or canaliculi and so this form is called *intra canalicular fibro adenoma*. It is the commonest variety of adenoma of the breast. When the fibrous stroma simply grows round the tubules the tumour is called *peri canalicular fibro adenoma*. The two forms often exist in the same tumour.

Involuntary muscle may form the stroma of an adenoma and then it is called *myo adenoma* as in the prostate. If the tubules of an adenoma are wide it is called a *cystic adenoma* or *cyst adenoma*. Such adenomata are common in the ovary. There are two types of ovarian cyst adenoma. The *pseudomucinous cyst adenoma* is usually found to have completely replaced the ovary. It is usually multilocular and may reach an enormous size. The loculi have thin walls of fibrous tissue rich in small spindle cells, contain a viscid fluid (pseudomucin) and are lined with tall columnar cells which have a pale vacuolated cytoplasm frequently distended by pseudomucin and nuclei arranged neatly along the basement membrane. Because of

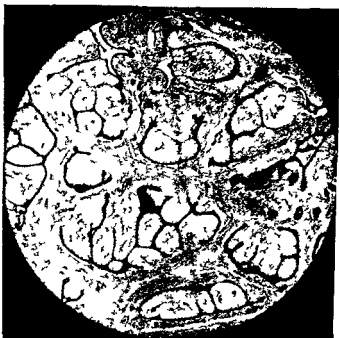


FIG. 33 —Intra canal ular Fibro adenoma of Breast Photographed with 14 cl Objective

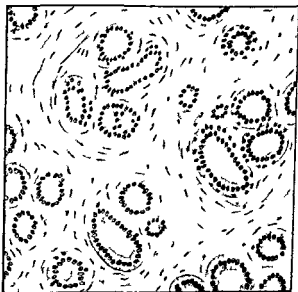


FIG. 34 —Peri canal ular F bro-adenoma of Breast Drawn under $\frac{3}{8}$ inch Objective

the histological resemblance of these cysts to the mucosa of large gut the occasional presence of involuntary muscle in the stroma and the less frequent association with dermoid cysts it has been suggested that they are teratomatous in origin. Less likely explanations of their origin are that they arise from the germinal epithelium or from the follicles. The *epoöphoric cyst adenoma* is usually unilocular contains thin watery fluid and is lined with cubical or short columnar epithelium. Frequently but not always it shows papillary projections on the inner surface these have a club shaped or dendritic core of fibrous tissue covered by a single layer of epithelium. The epoöphoric cyst is known as the papilliferous cyst adenoma of the ovary but as it has not always got papillæ and as it arises in the epoöphoron near the hilum of the ovary it is best called epoöphoric. The remnant of ovary is often found flattened out in its wall. It is also known as serous cyst adenoma on account of its thin serous fluid content. Some authorities assert strongly that it arises from germinal epithelium. Both the pseudomucinous and epoöphoric cyst adenomata may become malignant. The epithelial cells are then arranged to a greater or less extent in several layers the regular orientation of their nuclei being lost and vary in size and shape. The other forms of ovarian cyst are follicular lutein chocolate and dermoid. The *follicular cyst* is frequently multiple and usually small but occasionally reaches the size of an orange. It contains thin clear fluid and is lined by one or more rows of small spheroidal cells with scanty cytoplasm and shows a theca interna containing interstitial cells. The *lutein cyst* has a similar lining but the theca interna is occupied by numerous large polygonal lutein cells. The *chocolate cyst* may reach the size of a grape fruit it contains chocolate coloured or black altered blood it develops as the result of menstruation in areas of ectopic endometrium that appear in the ovary and is sometimes called an *endometrioma*. The endometrial lining has usually been destroyed extensively or completely and is replaced by a layer of large phagocytes loaded with pigments derived from hæmoglobin. Any remnants of endometrium consist generally of a layer of cubical or columnar possibly ciliated cells lying upon a narrow zone of endometrial stroma rarely small areas of glandular endometrium can be seen in the neighbourhood. The *dermoid cyst* is a teratoma.

Malignant Epithelial Tumours

These are the **carcinomata**. The malignant epithelial cells are the essential element in the tumours, but as the cells proliferate and penetrate body tissues they produce a reaction on the part of the host which provides a stroma of vascularised connective tissue. The cancer cells lie in groups in this stroma. Usually there is an infiltration of the stroma with lymphocytes, eosinophils and plasma cells.

Various points in their histological structure are made use of in the classification of carcinomata —

(1) Prevailing type of cancer cell in a growth. Squamous celled, squamous and horny celled, basal celled, spheroidal celled, polygonal celled, cubical celled and columnar celled carcinomata.

(2) Manner of arrangement of the cancer cells. Tubular, solid acinar, trabecular, papillary and papillary cystic carcinomata.

(3) Amount of stroma. Medullary and scirrhous carcinomata.

(4) Nature of chemical substance found in the carcinoma. Pseudomucinous or mucous carcinomata.

Squamous celled carcinoma. This growth is frequently called epithelioma. It may arise in any place where there is squamous epithelium and also occasionally, by a tissue change called metaplasia, in places where normally the epithelium is not squamous, *e g*, gall bladder, bronchus, breast and stomach. It frequently develops as a sequel of long-continued irritation, such as X rays on skin and stones in gall bladder or renal pelvis.

On the skin and other surfaces it is composed of long branching processes of squamous epithelium which grow downwards and destroy underlying tissue. Thus in the tongue the muscularis is usually invaded. The epithelial cells of which the processes are composed are not so uniform in size, shape and arrangement as in the benign papilloma and the normal epidermis. The variation in size, shape and staining reaction of the epithelial cells and their nuclei is usually conspicuous, very large cells with large deeply stained nuclei are frequent, multinucleate examples are usually present. The regular limiting layer of basal cells is frequently absent, especially from the most deeply seated processes. The processes

may indeed be very poorly limited and about them there may be small isolated groups and strands of spheroidal or polygonal epithelial cells. Prickle borders are absent to a greater or less extent. Groups of concentrically arranged horny scales and nucleated horny cells the so-called horny



FIG 35—Squamous Celled Carcinoma of Tongue Drawn under $\frac{1}{4}$ inch Objective

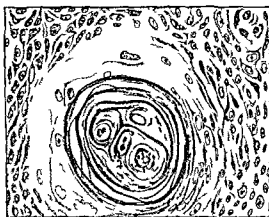


FIG 36—Horny Pearl or Cell Nest Drawn under $\frac{1}{4}$ inch Objective

pearls or cell nests are usually found irregularly distributed within the processes.

Rodent ulcer is a basal celled carcinoma i.e. a carcinoma composed of cells resembling those of the basal layer of the epidermis. The chief site of origin is the skin especially the skin of the face. The growth consists either of narrow

anastomosing processes or of large club like processes. The processes are composed of oval or polygonal cells bounded externally by a single row of regularly arranged cubical or columnar cells. The cells are small and have a scanty basophil protoplasm and an oval deeply stained nucleus. Frequently



FIG. 37 —Rodent Ulcer. Drawn under $\frac{1}{8}$ inch Object ve

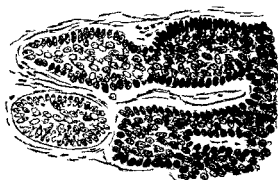


FIG. 38 —Rodent Ulcer. Drawn under $\frac{1}{12}$ inch Object e

portions of the stroma surrounding the processes are composed of jelly tissue being greatly rarefied and permeated by threads and droplets of mucus.

Tubular columnar or cubical celled carcinoma. This is often called adeno-carcinoma. Carcinoma of the large gut is nearly always of this type. It is usually ulcerated. In the

base of the ulcer all the layers of the gut wall are replaced by a cancerous tissue composed of tubules disposed irregularly in a fibrous stroma which is infiltrated by round cells. The tubules are very irregular in size and shape and are lined by deeply stained columnar and cubical cells with dark rod shaped nuclei which lie at all levels in the cells. Similar carcinoma of the small gut is very rare.

Solid acinar or alveolar polygonal celled carcinoma. This is the commonest type found in the breast. It is often called carcinoma simplex because of its simple structure but this is confusing because the term carcinoma simplex has also been



FIG 39—Columnar Celled Carcinoma of Colon. Drawn under $\frac{1}{2}$ inch Objective.

used to denote a carcinoma containing a moderate amount of fibrous stroma and thus lying midway between scirrhous and medullary carcinoma. The cancer cells are usually polygonal in shape but may be spheroidal cells. They are large, lightly stained and have a sharply marked cytoplasmic border. They are arranged in more or less rounded solid masses (solid acini or alveoli). Areas of trabecular carcinoma occur where the carcinoma cells are in narrow strands.

In a scirrhous carcinoma the fibrous stroma is abundant and dense in the centre of the growth but may be less abundant in the periphery. The cancer cells are usually arranged in small groups and narrow strands.

In medullary carcinoma there is very little stroma between large groups of cancer cells which are usually spheroidal.

Mucous carcinoma is found in the large gut, stomach, gall bladder and breast, as well as in other sites. The characteristic point about it is that mucus, frequently in large quantity, is formed by the secretion or disintegration of the cancer cells, so that sparsely scattered spheroidal or columnar epithelial cells float in a matrix of very pale, slightly hæmatoxyphil faintly filamentous mucus, sparse fibrous tissue septa traverse the growth. The mucus is stained bright red with muci carmine stain. It is this mucous carcinoma that is wrongly called "colloid" carcinoma. Pseudomucinous carcinoma is seen in the malignant form of the pseudomucinous ovarian cyst.

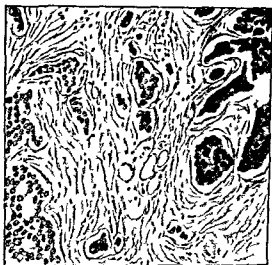


FIG 40 —Polygonal Celled Carcinoma of Breast.
Drawn under $\frac{1}{4}$ inch Objective

Papillary carcinoma is the malignant form of the papillary adenoma and may resemble it closely, but the epithelial cells covering the papillæ are irregularly arranged, frequently lying in several layers, and vary in size and shape, whilst the tissue beneath the papillæ is usually infiltrated by the epithelial cells. This sort of carcinoma occurs in the ovary arising from the epoöphoron, in the rectum and in the breast.

While the above list is useful as a classification of types of carcinoma, it must be recognised that many of these types may be found together in the one carcinoma. It is quite common in a cancer of the stomach to find areas of solid acinar, spheroidal celled growth, tubular areas, mucous areas

and both scirrhous and medullary areas. The breast is another place where multiplicity of type is found. In some sites, such as the large gut and body of uterus, carcinomata tend to be more constant in type. The same is true of the kidney, where far the commonest carcinoma is a growth known as *Grawitz' tumour*. This is composed of elongated alveoli lined with tall goblet like cells, alveoli into which project papillary processes covered with similar cells, and solid rounded masses of polygonal cells. The cytoplasm of the cells is loaded with fatty substances and glycogen, and is consequently remarkably clear and dropsical in a paraffin section. There are areas of dense hyaline oedematous fibrous tissue. One theory of the origin of this tumour is that it arises in the little nodules of accessory suprarenal cortex that are so commonly found at post-mortem in kidneys and under this theory it is called an *ectopic hypernephroma*. The majority of pathologists believe that the tumour arises from the epithelial cells of the kidney and that this name is a misnomer. The secondary growths from this tumour have a very similar structure. Such secondary growths are occasionally removed from the skin or other site and sent to the pathologist before the presence of the primary is suspected.

Benign Connective Tissue Tumours

This group comprises fibroma, lipoma, myoma, neuroma, gloma, osteoma and chondroma.

Fibroma. *Fibromata* may occur anywhere in the body, but are rare except in the ovary, skin and kidney. The renal fibroma is rarely larger than a pea. Fibromata are composed of spindle cells and a relatively large amount of collagen fibres arranged parallel to one another in bundles, the bundles being interwoven and seen cut in all directions in a section, well formed blood vessels pervade the growth. These tumours are not usually encapsulated nor is the border well defined from the surrounding tissue. An *epulis* is a generic term given to any nodule projecting from the gum. One variety is the fibrous epulis, composed of dense fibrous tissue with usually groups of plasma cells and other round cells. Some regard it as a chronic inflammatory nodule, others as a fibroma. The common *nasal polyp* used to be regarded as an oedematous fibroma, but is probably chronic inflammatory in nature. It is a lobulated projecting mass composed of a very rarefied

œdematous fibrous tissue, containing numerous mucous glands and densely infiltrated with lymphocytes, plasma cells and eosinophil leucocytes

Lipoma Lipomata are masses of adipose connective tissue, usually sharply defined and easily shelled out. Their structure is identical with that of normal adipose tissue. They sometimes contain areas of dense fibrous tissue and then the term fibro lipoma is used.

"Myxoma" The myxomatous tumours should all be regarded as malignant. (*Idem* myxo-sarcoma.)

Myoma A benign tumour of voluntary muscle is a *rhabdomyoma* and is a great rarity, while one of involuntary

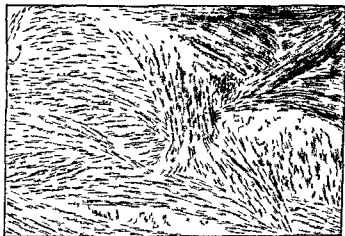


FIG. 41.—Fibroma of Ovary. Drawn under $\frac{1}{2}$ inch Objective.

muscle is a *leiomyoma* and is a very common tumour. Leiomyomata occur most commonly in the uterus and occasionally in the broad ligaments, they are frequently called *fibroids*. They are composed of typical involuntary muscle fibres arranged in bundles which are whorled and interwoven. They are often called fibro myomata because a greater or less amount of collagenous fibre lies between the muscle cells. The muscle fibres are long narrow cells with rod shaped nuclei, the cytoplasm is stained reddish purple in hæmatoxylin and eosin preparations, and yellow with Van Gieson's stain. In transverse section each fibre is polygonal, has a central nucleus and lies in a polygonal space bounded by a delicate collagenous fibril.

Leiomyomata also occur in the stomach, usually as small tumours, and in the skin, probably arising from the erecting muscles of the hairs

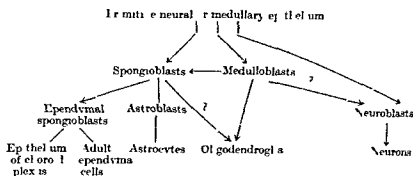
Degenerative changes occur commonly in fibro myomata and fibromata, and to a less extent in lipomata as the result of interference with the blood supply of the tumour Hyaline fibrous degeneration consists in the collagen fibres becoming very dense and tending to fuse together fibroblasts and muscle fibres decreasing When necrosis occurs whole areas are seen devoid of nuclei or with nuclei in a state of chromatolysis The so called red degeneration is a combination of partial or complete necrosis and hæmolytic staining, the hæmoglobin or its decomposition derivatives being diffused through the tumour Calcification results in part or all of the tumour being converted into a calcareous mass Edema is common and then the growth is sometimes called *pseudo myoma*, a cyst may develop as the result of an area of extreme oedema Leiomyosarcoma sometimes arises in a leiomyoma

Glioma Glioma is the commonest primary tumour of the brain but is rare in the spinal cord The differentiation between glioma and gliosarcoma is very difficult is generally evaded and is of no practical importance because both are equally fatal owing to their situation and glial tumours very rarely give rise to metastasis

Glial tumours vary greatly in the relative number of cells and glial fibrils and in the form of the glial cells Some are composed almost entirely of small round cells in others cells of a great variety of shapes and usually furnished with branching processes are associated with a moderate amount of fibre in others astrocytes are embedded in a dense fibrillar matrix Glioma and gliosarcoma are examples of tumours of *epiblastic connective tissue*

The use of silver and gold impregnation and other special methods of staining sections is necessary for Bailey and Cushing's classification of gliomata In the following brief account of this classification it is to be noted that it includes tumours that are not strictly glial tumours It comprises all tumours of the central nervous system of epiblastic origin The classification depends on the resemblance of the cells comprising the different varieties of tumour to the cells composing the central nervous system at the different stages of

its development A list of the latter may be represented as follows —



Thus a glioma composed of cells like the primitive neural or medullary epithelium is called a *medullo epithelioma* but is a very rare tumour. There is no sharp demarcation between *ependymoblastoma* and *ependymoma*. In ependymal tumours there is variation in the proportion of more cellular and more fibrillar areas. In the more cellular areas the cells are polygonal and some of them are arranged to form tubule like spaces. Minute bodies called blepharoplasts are usually found as in normal ependyma in the cytoplasm near the border of the spaces when cilia are also present or the blepharoplasts may be grouped in a clear halo nearer the nucleus. The more fibrillar parts are composed of interlacing bundles of fibrillated long spindle cells with blepharoplasts near the nucleus. Owing to the difference in the areas a faintly lobulated appearance is appreciable with the naked eye. The tumours are firm and usually circumscribed. They usually arise near the ventricles in the brain and near the central canal in the cord. They are relatively slow growing gliomas. *Papilloma of the choroid plexus* is usually found projecting into one of the ventricles. Its branching villous processes are composed of cores of mesoblastic (collagenous) fibrils and fibroblasts covered with columnar cells without blepharoplasts and without cilia.

Medulloblastoma is a soft greyish white tumour composed of closely packed undifferentiated round or carrot shaped cells and microscopically might easily be mistaken for a round-celled sarcoma. But in it are found solid rosettes which are small spherical bodies whose peripheral zone is made up of small round or oval nuclei surrounding a central

zone of interwoven thread like cytoplasmic processes. In some of the more slowly growing tumours areas of feebly fibrillated polar spongioblasts are present. This is interpreted as an attempt at differentiation. Medulloblastoma most commonly occurs in the vermis of the cerebellum in children and as might be expected from the undifferentiated nature of its cells its growth is relatively malignant and rapid. It often spreads to form a thin sheet in the pia arachnoid macroscopically simulating meningitis. Glioma (neuroepithelioma) of the retina may also spread in this way and is of very similar



FIG 42—Ependymoma $\times 10$
H and E



FIG 43—Medulloblastoma showing
rosettes $\times 94$ H and E

structure. Both tubular and solid rosettes have been found in the primary tumour.

A very common form of glioma is *spongioblastoma multiforme*—a soft often hæmorrhagic and necrotic rapidly growing tumour found usually in the cerebrum of adults. Its cells vary greatly in size and shape pyriform and spindle (spongioblastic) types and multinucleate giant cells being very common. Palisading of spongioblast like cells around necrotic areas is very characteristic. Another common variety is *astrocytoma*. This may be either of the small cell type in which small stellate cells are rather sparsely and evenly scattered through a dense feltwork of interlacing

neuroglial fibrils, or may be composed of more closely packed and larger stellate and polygonal cells with heavily fibrillated

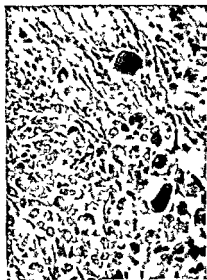


FIG 44 —Spongioblastoma multiforme
× 166 H and E

processes. One of the stout processes of these stellate astrocytes is attached to a blood vessel, and is called the "sucker foot." These growths occur anywhere in the brain. They may be soft and cystic or hard and very rubbery. They are of relatively slow growth.

Oligodendroglioma is a relatively uncommon type of glioma. It is usually found in the cerebral hemispheres of young adults. A zone of calcified particles is frequently found at its periphery, and is seen as a crenated line in X-ray photographs.

Microscopically a very characteristic picture is seen in which groups of closely packed round or polygonal



FIG 45 — Fibrillary astrocytoma
× 186 Phosphotungstic acid
hematoxylin

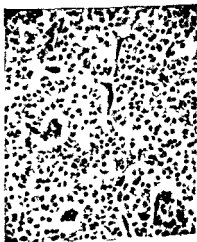


FIG 46 —Oligodendroglioma, × 166
Phosphotungstic acid hematoxylin

cells; with round central nucleus and clear, empty looking cytoplasm, are enclosed by trabeculae of collagenous connective tissue containing blood vessels. This vacuolated appearance of the cells is a result of degeneration ("acute swelling"), to which oligodendroglial cells are particularly susceptible. By specific silver impregnation methods, however, the typical cells with processes can often be demonstrated, especially near the periphery of the tumour. *Ganglioneuroma* and *neuroblastoma* are exceedingly rare in the central nervous system.

Neuroma *Ganglioneuroma* and *embryonic neuroma* are rare tumours. They occur in the medulla of the suprarenal body and in the sympathetic ganglia. The *ganglioneuromata* contain ganglion cells and either fully developed or embryonic nerve tissue. The *embryonic neuromata* are apparently identical with the *medulloblastomata* previously described. They consist of small round and oval cells and a variable quantity of delicate fibrils suggesting glia, a characteristic is the presence of solid rosettes. The commoner neuroma is the *fibrouneuroma* or *neurinoma* or *molluscum fibrosum*. These *neuromata* are generally found in the skin or upon nerves, and multiple examples in both positions are found in von Recklinghausen's neurofibromatosis. They occur sometimes on cranial or spinal nerve roots, those of the eighth cranial nerve being called "*acoustic tumours*". They consist for the most part of interlacing bundles of very long spindle cells or of elongated cylindrical fibres furnished with nuclei at intervals, closely connected with collagenous fibrils. They are thus very similar to leiomyomata and some endotheliomata. In contrast to leiomyoma the fibres are round instead of polygonal and the nuclei are sparser. The differentiation can seldom be made with certainty unless cross sections of some bundles are found to be occupied by sections of tubes with a thin collagenous wall and a nucleus projecting into the tube *an axis cylinder may be within the tube but this is very rare*, the majority of the tumours being apparently formed by cells of the sheath of Schwann alone—*neurinomata*. The tubes tend to become solid hyaline fibres and much of the tumour may be composed of collagenous fibrous tissue. A characteristic structural feature sometimes seen in these tumours is called "*palisading*", areas where nuclei are arranged side by side in a row are separated by areas of fibres.

devoid of nuclei. *Amputation or traumatic neuroma* occurs on the cut end of a nerve and is composed of numerous bundles of typical nerve fibres formed in an attempt at regeneration.

Chondroma Tumours of cartilage are divided into *enchondromata* and *ecchondromata*. The distinction according to some writers is that an *enchondroma* develops in a site where cartilage is normally found and an *ecchondroma* in a site where cartilage is not normally found. It is more convenient to define an *enchondroma* as one which arises inside a bone and an *ecchondroma* as one which projects from a bone or cartilage. Wherever they occur they are composed of hyaline cartilage separated into rounded lobules by fibrous tissue containing vessels. Between the fibrous tissue and the cartilage there is usually a single layer of cells, the cellular layer of the perichondrium. The cartilage differs from the normal in that the cells tend to be larger, fewer and more irregularly disposed, whilst the matrix tends to be hyaline and is very often apt to contain small or large mu areas, so the term *myxochondroma* is used. Areas of the matrix may be calcified, the calcium appearing as deeply hæmatoxylin granules. Bone may be formed by the ordinary process of endochondral ossification and the tumour is then called an *ossifying chondroma*. The *chondrosarcoma* is characterised by an abundance of cells, sometimes showing great variety in size and shape in the cellular layer of the perichondrium. *Chondromata* in which this characteristic is absent have however been known to give rise to metastasis.

Osteoma The commonest osteoma is an *ossifying ecchondroma*. It usually projects from a long bone close to an epiphysis. It is made up almost entirely of cancellous bone with marrow, but has an external cap of cartilage of variable thickness. *Membranous osteoma* occurs more rarely, usually in the skull and in this variety the bone is formed by the periosteum. It may be either a *cancellous* or an *ivory osteoma*. An osteoma is sometimes called an *exostosis*.

Malignant Tumours of Connective Tissue

These are cellular or pure sarcoma, myxosarcoma, leiomyosarcoma, chondrosarcoma, neurosarcoma and gliosarcoma.

as the ¹st or pure sarcoma. These are 1

are composed of densely packed cells with very little intercellular tissue. They are tumours in which the cells have not differentiated into any sort of connective tissue, such as the fibrous tissue of bone from which they arise. They are classified into small round celled, large round celled, spindle celled and mixed celled.

Small round celled and large round celled sarcomata These are not common tumours. They are found chiefly in the periosteum and intermuscular fascia. They are composed of small or large round cells closely set together. The

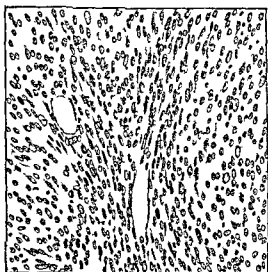


FIG. 4 —Spindle Celled Sarcoma. Drawn under $\frac{1}{4}$ inch Objective

nucleus has an abundant net of delicate fibrils of chromatin, numerous small nodes and inconspicuous nucleoli. The cytoplasm is scanty and ill defined. Vessels with muscular walls are absent.

Spindle celled sarcoma 'Pure or undifferentiated spindle celled sarcoma is not a common tumour. The cells have oval or spindle nuclei, scanty cytoplasm and poorly demarcated cell outline. They are arranged parallel to one another in bundles which are interwoven, so that in a section they are cut in all directions.

Mixed celled or polymorphic celled sarcomata are composed of cells of all varieties of shape.

These "pure" or very cellular undifferentiated sarcomata are rapidly growing and very malignant tumours. They often contain very numerous mitotic figures. Owing to the delicately cellular nature of the tissue and to its having only thin walled blood vessels, hæmorrhage and necrosis is common and extensive. In other sarcomata the tumour cells manufacture more or less connective tissue of various sorts, according to the type of connective tissue cell from which they arise. The more they differentiate into connective tissue, the less malignant are they likely to be. Sarcomata are frequently composed of areas of differentiated tissue mixed with areas of more cellular undifferentiated tissue, the latter being most frequently found in the peripheral part of the tumour.

Fibrosarcoma is found especially in skin, subcutis, intermuscular fascia and retroperitoneal tissue. It is composed of spindle cells and collagen fibres arranged in bundles. The cells tend to differ in size and shape, and some of them may have several nuclei. Areas with more cells and less fibres may be present, usually at the growing edge. The blood vessels have thin walls. Tumours composed of quite dense fibrous tissue may yet be surely, if slowly, malignant and show conspicuous invasion of tissue. "*Desmoid*" is a name given to a slow growing fibrosarcoma of abdominal wall.

Neurinosarcoma or neurofibrosarcoma is the malignant form of fibro-neuroma and resembles it in structure, but in areas the nuclei are closely packed, vary in size and staining reaction, and have many mitotic figures. It may be very difficult to distinguish it from ordinary spindle celled sarcoma or fibrosarcoma.

Gliosarcoma has already been referred to under glioma.

Myxosarcoma is found in the same sites as fibrosarcoma and chondrosarcoma. Myxosarcoma may constitute the whole of a tumour or it may form part of a fibrosarcoma (myxo-fibrosarcoma) or part of a chondrosarcoma (myxo-chondrosarcoma). It is also the commonest sarcoma of the breast, but here usually arises from the stroma of a fibro-adenoma. It is composed of a very rarefied network of spindle, stellate and multinucleate cells and collagen fibres, in the meshes of the net is material which appears as a very pale matrix on examination with low magnification, but with higher magnification shows delicate faintly hæmatoxyphil threads.

and beads this material is stained red with mucicarmine stain

Leiomyosarcoma, or sarcoma developing from unstripped muscle, occurs most commonly in the uterus, usually arising in a fibromyoma. In parts of the tumour moderately typical involuntary muscle fibres are seen with little collagen fibre, in other parts the cells vary greatly in size and shape multi nucleate cells with a broad strip of cytoplasm like a muscle fibre and large irregularly clumped frequently deeply stained nuclei being a characteristic feature

Chondrosarcoma Malignant tumours composed entirely of cartilage have been already described in dealing with chondroma

Osteo chondrosarcoma This is often called osteosarcoma. It is the commonest form of periosteal sarcoma. It arises more rarely in endosteum. Although there may be considerable differentiation of tumour cells into bony and cartilaginous tissue yet there are usually also areas of very cellular sarcoma. The tumours are very malignant. The most characteristic examples show the following layers in a section taken at right angles through the fusiform swelling of the periosteum. a peripheral zone rich in round spindle or polymorphic cells with little intercellular tissue a deeper zone containing collagen fibres which in areas become hyaline and fuse to form a homogeneous matrix containing encapsulated cartilage cells in other areas the collagen fibres fuse to form trabeculae of membranous bone which are calcified to a greater or less extent. The bony trabeculae may radiate from the periosteal surface of the bone. The Haversian canals of the cortex are pervaded by growth but the contour of the cortex is usually little disturbed. the medullary cavity may also contain growth but is not widened. A sarcoma of a similar microscopical structure can arise from endosteum and the indication of this difference in origin is given by expansion of the medullary cavity

Myeloid sarcoma arises from the periosteum of the jaw and the endosteum of other bones. In the bones it forms a soft rounded mass which is bounded by a thin bony capsule continuous with the neighbouring corticals. The tumour contains numerous giant cells which tend to be very large have a large mass of scoriaceous cytoplasm and contain many scattered oval nuclei, between the giant cells are oval and

stellate cells like fibroblasts and a little collagen fibre hæmorrhage in the tumour is frequent. Bony trabeculae are frequently formed in the periphery of the tumour, but very seldom within it. As the neoplasm does not infiltrate but rather compresses surrounding tissue and practically never gives rise to secondary growths objection is made to its being called "sarcoma". The giant cells resemble the normal osteoclasts of bone so it is sometimes called osteoclastoma. It is also called a myeloma (a tumour of marrow) but if so

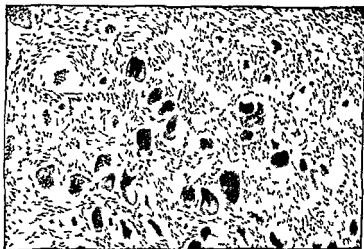


FIG. 48.—Osteogenous or Giant Celled Myeloma. Drawn under $\frac{1}{3}$ inch Objective

should be named **osteogenous myeloma** because it arises from the bone forming connective tissue of the marrow.

The myeloid sarcomata (osteogenous myelomata) of the gum project from the alveolar margin and form one kind of epulis. They have the same structure as the osteogenous myelomata of long bones.

The hæmatogenous myeloma takes its origin from the blood forming tissue of the marrow. It occurs as multiple confluent soft tumours which arise within the medulla of the vertebrae and long bones and may infiltrate the periosteum and adjacent soft parts. The condition is sometimes called *multiple myeloma* or *myelomatosis*. A special substance called Bence Jones' albumose frequently appears in the urine of the patients. The majority of the tumours are composed of small round cells which bear a superficial resemblance to plasma cells,

others are composed of differentiated myelocytes or, very rarely, erythroblasts

Melanoma

These are neoplasms containing melanin pigment. They are benign or malignant.

The **benign melanoma** is found in the skin, where it may be present at birth, and is known as a pigmented naevus or hairy mole, or it may appear after birth. The cells lie in the dermis, are spheroidal or oval and tend to be small and uniform in size. They are arranged diffusely in places but a characteristic



FIG. 49—Malignant Melanoma. Drawn under $\frac{1}{2}$ inch Objective

feature is the occurrence of solid acinar groups of cells usually just under the epidermis. melanin pigment is sometimes scanty, and then is usually found in the cells just under the epidermis.

The **malignant melanoma** arises in the skin or choroid. In the skin it used to be called melanotic sarcoma, but now some authorities think it is a carcinoma arising from the epidermis, and probably from the basal layer. The cells are spindle and spheroidal, but tend to vary greatly in size and shape and may be multinucleate, their cytoplasm may be scanty or abundant, the acinar grouping is again seen in places suggesting a carcinomatous structure. Melanin pigment varies greatly in amount in different examples, it is for the most part within the cytoplasm of tumour cells.

The *choroidal melanoma* is a malignant tumour, but the appearance of the secondary growths may be delayed for several years. It is found as an oval mass arising in the choroid and frequently projecting the retina forwards into the vitreous. The cells are round and oval, and usually contain much pigment.

Endothelial Tumours

Endotheliomata arising in serous surfaces Tumours arising from the endothelial cells of the serous surfaces are very rare and usually malignant. They are occasionally found in the pleura and peritoneum and resemble carcinomata in structure, being composed of spheroidal, polygonal and oval cells arranged in groups in a fibrous stroma.

Endotheliomata of vascular endothelium These are tumours arising from the endothelium of blood or lymph vessels. They may be benign or malignant. The benign form is called *angioma*, and may be *lymphangioma* or *hæmangioma*.

Lymphangioma is comparatively rare. It occurs in the connective tissue of the neck as a congenital tumour called *cystic hygroma*. It is found elsewhere in the subcutis. It is made up of somewhat wide spaces, containing clear white fluid lined by flat endothelial cells and bounded by fibrous tissue which occasionally contains a few involuntary muscle fibres. Irregular areas of lymphatic gland tissue are frequently scattered through the growth.

Hæmangioma is very common in the liver, dermis, subcutis and intermuscular fascia. The tumours are frequently congenital, being present at birth, and some of them show little or no increase in size. They are divided into *cavernous hæmangioma* and *capillary hæmangioma*, according as the blood spaces of the tumour are large or small. The former has wide spaces lined by a single layer of flat endothelium and walls composed of fibrous tissue. The spaces contain blood which may clot and organise, turning a part or the whole of the tumour into a scar. The capillary hæmangioma is much more cellular, the oval nucleated endothelial cells and collagen fibrils tend to develop in concentric whorls, in the centre of which are small blood spaces, larger blood spaces also are found. Either variety of hæmangioma may have an ill-defined outline, lobules of

tumour tissue lying, for example, between the fibres of voluntary muscle

An important tumour is the fibro-endothelioma of the cerebral dura. It arises as a sessile mass from the inner surface of the dura, usually over the vertex. It frequently invades the skull, causing local thickening of the bone, but rarely invades the brain, being easily shelled out from the indentation it has made in the cerebral cortex, though its removal during life may be attended with great hæmorrhage. It is composed of oval and spindle endothelial cells and collagen fibrils, frequently arranged in concentric whorls. It arises in the dura from the endothelial cells of the small arachnoid villi that normally penetrate the dura.

True angioma is much rarer in the central nervous system. Capillary angioma is rare in the leptomeninges, but is commoner in the brain substance, especially the cerebellum. Cavernous angioma occurs occasionally in the cerebrum. Both are very rare in the spinal cord. Progressive dilatation of congenitally malformed vessels forming an area of wide blood spaces (*serpentine aneurysm*) is found in the leptomeninges, and may implicate and destroy the substance of brain or cord.

Malignant endothelioma arising from vascular endothelium is a rare tumour. It is composed of irregularly disposed, frequently atypical, endothelial cells, and the diagnosis from sarcoma may be very difficult, a tendency towards the formation of blood spaces by the tumour cells is frequently seen, while the recognition of the endothelial character of the cells is a matter for the more expert cytologist.

Teratomata

A teratoma arises from "cell rests" that occur at an early period in embryonic life. The cells of these "rests" can form tissues derived from all three germinal layers—epiderm, mesoderm and hypoderm. Adam called them "toti potent" cells.

The commonest teratoma is the ovarian dermoid cyst. It tends to replace the ovary, is usually unilocular and contains butter-like sebaceous matter and hair. It is lined chiefly by skin with many sebaceous glands and perhaps sweat glands. If many sections are taken from its wall, irregular areas of

fully differentiated tissues and portions of organs are found mixed together, and it is an interesting and salutary exercise in normal histology to attempt their recognition. Thus bone cartilage, adipose tissue, glia and columnar tubes like bronchi are commonly found, thyroid, portions of stomach and intestine and fully formed teeth are not rare. The majority of these tissues will be found in a globular mass that usually projects into the interior of the cyst. The ovarian dermoid is benign, but may give rise to squamous carcinoma. The commonest tumour of the testis is a teratoma, but it very rarely shows such an admixture of well differentiated tissues as the ovarian dermoid. Well formed cartilage is frequently found, but the greater part of the tumour is often composed of large round cells and very poorly differentiated embryonic tissues of carcinomatous and sarcomatous appearance. The tumours are very malignant. Teratomata occur rarely in other parts of the body.

Teratoblastomata

These mixed tumours arise from cells that are capable of giving rise to tissues derived from one or two of the primitive germinal layers, but not from all three. Adam named such cells "pluri potent."

The commonest teratoblastoma is the *mixed tumour of salivary gland*. It is particularly found in the parotid, but also in the submaxillary gland and in the palate. It usually has a well defined border. It is composed of myxomatous tissue and tissue having a close resemblance to cartilage, scattered about in this stroma there are solid masses of polygonal epithelial cells and tubules formed by cubical epithelium. The tubules contain a hyaline eosinophilous material. Areas of squamous and horny epithelium are sometimes present. Some authorities deny that this tumour of salivary gland is a teratoblastoma and say it is an adenoma, asserting that the tissue resembling cartilage is not true cartilage, but is developed as a change in the mucoid connective tissue, the mucoid material in the connective tissue being secreted by the epithelial cells. Malignant carcinomatous varieties occur. A teratoblastoma is found in the region of the kidney of infants. It is a malignant tumour composed of epithelial cells and tubules and of fibro, myxo, and myo sarcoma.

Teratogenous or Heterochthonous Blastomata

This group comprises blastomata which do not arise from the cells of the host but from the cells of a parasitic individual —e.g., a foetus in utero or dermoid cyst

Hydatidiform mole This is a tumour of chorionic epithelium. It therefore arises from cells belonging to the embryo and not from the tissues of the mother. The connective tissue of the centres of the chorionic villi becomes very oedematous and the villi are converted into cysts. The epithelial cells of Langhans' layer and the syncytium proliferate to a greater extent than in normal chorionic villi and are atypical. Such excessive and atypical proliferation is usually seen in focal masses upon villi. There is no sharp histological difference between this atypical growth and that which leads to chorion carcinoma.

Chorion carcinoma is a malignant neoplasm of chorionic epithelium and frequently follows hydatidiform mole. The wall of the uterus is found to be infiltrated by very atypical cells. Some of these cells are very large and multinucleate suggesting syncytial masses. There is much hæmorrhage in the infiltrated tissue. The secondary growths are largely composed of hæmorrhage and clot similar polymorphic and multinucleate cells being confined mainly or entirely to the periphery. Chorion carcinoma occurs in the testicle taking origin here from a teratoma.

CHAPTER XXVII

HISTOLOGICAL METHODS

THE tissues removed at operation are preferably received into the laboratory immediately after removal without the addition of any preserving fluid. The constant handling of tissues gives the pathologist a considerable acquaintance with their various appearances and the naked eye observation is of great assistance to him. The addition of alcohol or formalin to the tissues alters their appearance. If for geographical or other reasons a preservative is necessary the tissues should be placed in 4 per cent saline formaldehyde (p 462). If formalin is not available it is better to use methylated spirit *or any sort of alcohol than to allow small portions of tissue to become dry or than to leave them for more than a few minutes in saline or water*.

On the other hand a further examination with the naked eye after the tissue has been in formaldehyde solution for some hours or days will often reveal important features that may not have been noticed in the fresh specimen particularly in the case of a large very soft specimen.

It is most important that as much of the material should be obtained as possible in order that the relationship of the normal and abnormal tissues can be noted and portions removed from the most desirable places. Indeed it is best to make a rule that every scrap of tissue excised at the operation should be sent to the pathologist. It is for him to decide which are the parts that require microscopic examination. At the same time he should always try to comply with the surgeon's requests for examination of particular places.

In the case of small specimens where superficial and deep surfaces and cut edges are difficult to recognise with the naked eye it is imperative to obtain exact topographical details from the surgeon before deciding in which plane the section is to be made.

The pathologist should make and preserve records of naked eye appearances of all specimens, with details of measurement, colour and consistence of every part. Large specimens should undergo a careful dissection and investigation with many cuts, which should be made so that the different parts are not completely dislocated from their relations to one another, as it is often necessary to review a specimen after the microscopic sections have been seen. For instance, in all lumps or solid organs, such as the spleen, all cuts should be parallel to one another and should be so deep as to include almost the whole depth of the specimen. In a breast cuts should be made through the skin, not through the deep surface and should traverse the whole breadth and depth of the breast, the first cut should include the nipple and any tumour that may be present. Organs containing tubes or cavities must be opened and cut in ways suitable to their special structures.

The selection of the portions to be sectioned must be made with care. It is a common error to remove large areas of necrosed or very hæmorrhagic tissue for section. The necrosed and hæmorrhagic parts must be examined microscopically, but it is much more important to search for areas unaffected by such changes. Portions should be chosen from the fully developed central area of the lesion as well as from the margin, and the latter should include the junction of normal and abnormal tissue. Samples of all different varieties of pathological tissue should be taken for section.

A record should be kept of the exact sites and appearances of portions taken for section, accompanied sometimes by sketches of the portions.

All specimens however common and ordinary they may appear to the naked eye, should be kept labelled in reserve in about 2 per cent formaldehyde solution until the final microscopic diagnosis has been made. Even in the case of very small specimens if possible at least a minute fragment should be retained in formalin for reference. This comes in useful when, as sometimes happens, a suspicion arises that portions taken for section from different cases may have got mixed on the way from the naked eye examination to the microtome.

When the bits for section are small fragments of friable tissue, e.g., curettings, they are best placed in a piece of gauze tied with a thread in the form of a sac. The tissues can remain

in the gauze sac until the stage of "casting" in the paraffin process

Tissues containing calcareous areas or bone must be decalcified by a process described later, and it is advisable to examine separately by the ordinary method a soft portion, if such is available

The three chief methods of obtaining microscopic sections are the freezing method, embedding in paraffin and embedding in celloidin. The celloidin process is only required for a few special purposes and will not be described here

Frozen sections This method must be used if it is necessary to have sections in which the fats can be stained with Scharlach R or Sudan III, because fat is dissolved out of the tissue in the paraffin and celloidin processes by such solvents as absolute alcohol, chloroform and ether. It is also used for getting sections quickly

The apparatus required consists of a cylinder of CO_2 and one of the many types of freezing microtome made for use with that freezing medium. The gum solution that may or may not be used is made up as follows —

Saturate gum acacia in hot water

Mix 3 parts of the gum solution with 1 part of syrup (B.P.)

The preparation of sections to show fat. Leave a portion of tissue not more than 2 cm in diameter and not more than 0.3 cm thick for 12 to 24 hours or longer in 4 per cent saline formaldehyde, which is made up as follows —

Commercial formalin (40 per cent formaldehyde) 10 c c

0.9 per cent solution of sodium chloride in water 90 c c

Then it is perhaps wise to wash the tissue in running water for from 12 to 24 hours to try to get rid of the formalin, but this step may be omitted. Next, either soak the tissue in the gum solution for 24 hours place it on the stage of the freezing microtome, cover it with gum solution freeze it and cut sections, or take the tissue straight from the water or formalin to the stage of the microtome, freeze it and cut sections. Most tissues can be cut by the latter method but should the tissue tend to separate from the stage of the microtome during cutting then the gum solution may be used

Transfer the sections with a camel hair brush from the microtome knife to water and leave for a few minutes

Transfer to 70 per cent alcohol for a few seconds

Leave for 5 minutes in a well stoppered bottle containing freshly filtered Herxheimer's solution of Scharlach R made up as follows —

70 per cent alcohol	50 c c
Acetone	50 c c
Scharlach R	1 gram

Wash in 70 per cent alcohol for a few minutes till differentiation is visible between the red stained fat and the pale tissue

Wash in water for 1 minute

Transfer to any ordinary staining solution of hæmatoxylin *e.g.* Ehrlich's for 30 seconds

Wash in tap water till section is blue

Wash in weak acid alcohol (0.25 per cent hydrochloric acid in 70 per cent alcohol) for a few seconds till section is pink

Transfer again to tap water till blue

Pick section from the water on to the middle of a slide

Drain off excess of water and just before section loses all its moisture mount it in neutral glycerine jelly

The rapid preparation of sections This can be done in a few minutes by placing upon the stage of a freezing microtome a piece of tissue taken straight from the body at operation cutting sections and staining them rapidly. Such sections cut from tissues that have not been hardened in a fixing solution are apt to be thick and difficult to interpret unless the technician has great experience and skill. It is only on rare occasions that more information can be obtained from them than can be elicited by the surgeon or pathologist from the appearance of the specimen with the naked eye.

Thinner sections can be got with the freezing microtome after fixation for 10 minutes or more in 4 per cent saline formaldehyde the fixative containing the tissue being kept hot and agitated in a water bath at about 60° C.

Sections may be stained quickly in the following way —

Transfer section from water to slide

Remove excess of water from slide

Cover section with Ehrlich's hæmatoxylin and heat gently over a Bunsen burner for 1 or 2 minutes

Transfer section from slide to tap water for a few seconds

Leave in a solution of 1 part of a concentrated watery solution of lithium carbonate to 10 parts of water for a few seconds till section is blue

Wash in weak acid alcohol for a few seconds till pink

Rinse in tap water

Leave in lithium carbonate solution for a few seconds till blue

Stain with watery solution of eosin for 2 minutes

Rinse section in tap water for a few seconds, and if it is not getting pale red quickly enough, wash it in 70 per cent alcohol for a few seconds

Transfer section to a slide

Blotting section carefully helps to fix it on to the slide

Dip slide in absolute alcohol for a few seconds

Blot section

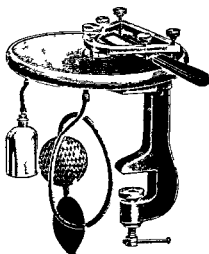


FIG 50—Williams Freezing Microtome

Dip it in xylol for a few seconds

Mount in Canada balsam

If CO_2 is not available or if a portable freezing microtome is required, then a convenient pattern is Williams' microtome (Fig 50) made by Messrs Swift and Son. Here the freezing is done with ether and ethyl chloride sprays. The microtome consists of a circular plate fitted with a clamp, which can be attached to the edge of a firm table, and of a freezing apparatus by means of which a spray of ether can be directed against the under surface of a small metal disc let into the circular plate. An ordinary razor blade is fitted into a brass tripod carrier at a fixed angle. The level of the cutting edge is regulated by a screw at the apex of the tripod. In the

slower method the portion of tissue is fixed in 4 per cent formaldehyde, washed in water and transferred to gum solution for 24 hours before being placed on the microtome. In the quicker method it is placed straightaway on the microtome.

The process then is as follows —

Put the tissue on the central disc of the microtome stand. Just cover it with gum solution. Do not pour on so much gum solution that it flows over the edge of the disc. Freeze with the ether spray from below, assisted by the ethyl-chloride spray from above.

The freezing is complete when the gum is quite white and the tissue firm to the touch. If the freezing process is continued too long the tissue becomes extremely hard and cannot be cut at all, in which case thawing may be hastened by moistening tissue and gum with warm water. If the freezing is insufficient the gum is readily dented with slight pressure of the finger and the tissue leaves its bed when the razor meets it.

The freezing process should be completed in a few minutes.

Moisten the stage of the microtome with water to allow the razor carrier to slide easily.

Adjust the level of the razor edge exactly to the height of the tissue.

Hold the razor carrier in both hands, with the forefinger of the right hand resting on the front adjusting screw.

Rapidly sweep the razor across the tissue, keeping the carrier legs pressed against the microtome stand surface.

With the forefinger turn the screw a short distance onwards to depress the cutting edge. The amount of the turn determines the thickness of the section cut.

Repeat the process about a dozen times in rapid succession.

With a small camel hair brush wipe gently the mixture of gum and tissue from the upper surface of the razor blade into a tall glass beaker filled with warm normal saline. The sections float out on the surface of the fluid and can be assisted to separate by gently touching with the brush.

If complete and thin sections do not appear, cut more either of different thickness or after altering the consistence of the gum and tissue by further freezing or thawing.

The sections can then be stained by one of the shorter or longer staining methods.

The preparation of paraffin sections. For this should be

provided a set of well stoppered wide mouthed, glass pots containing respectively 50 per cent alcohol, 70 per cent alcohol, 90 per cent alcohol, three pots of absolute alcohol, all, or at least one, of which should contain a layer of anhydrous copper sulphate covered with a filter paper for the portions of tissue to rest upon, a pot of chloroform, a pot of equal parts of chloroform and of paraffin wax which has a melting point of about 58°C . A paraffin oven kept at 60°C is also required.

Cut a piece of the tissue between 1 and 2 cm broad and as thin as possible provided it does not curl up, this usually means a thickness of about 3 or 4 mm.

Place it in ten times its own volume of 4 per cent saline formaldehyde for 12 hours.

Trim the now hardened tissue till it is not more than 3 mm thick.

For the identification later of the surface that is to be cut, a notch should be cut in the opposite surface.

Place the tissue in fresh 4 per cent saline formaldehyde for 12 hours or longer. The best results are obtained after 7 days' fixation, but such a time cannot usually be afforded when a diagnosis is being made during the life of the patient.

Transfer to 50 per cent alcohol for 12 hours.

Transfer to 70 per cent alcohol for 12 hours.

Blot the tissue with blotting paper or a clean cloth and transfer to 90 per cent alcohol for 3 hours.

(If, unfortunately, the tissue reaches the laboratory in methylated spirit, a portion can be transferred from the methylated spirit to absolute alcohol and the process carried on from that point.)

Put the tissue through the three pots of absolute alcohol, 1 hour in each, blotting the tissue between each change.

Leave the tissue in chloroform till it sinks below the surface, about 12 hours.

Transfer to the mixture of chloroform and paraffin wax for 30 minutes at room temperature and 30 minutes in an incubator at 60°C .

Put the tissue through three baths of wax on the paraffin oven, 1 hour in each.

The tissue is then ready to "cast" in a flat bottomed glass or aluminum dish about 1.5 or 2 cm deep, and of an area

suitable for the reception of the number of pieces ready for embedding

Smear the inside of the dish with glycerine, keeping the dish warm

Fill the dish about three quarters full of wax from the last bath and then quickly pick the tissue out of the bath with warmed forceps and press it gently down on the bottom of the dish the surface or edge that is to be cut going downwards then fill the dish to the top with wax

Blow on the surface of the wax till a skin forms Cover this skin with blotting paper that has been soaked in cold water

After a few seconds immerse the dish in cold water to harden the wax quickly

The hard block of wax can easily be separated from the dish and trimmed so that only a small margin of wax is left around the tissue

The reagents used in this process must be changed frequently especially the absolute alcohol

Sections can be cut at once with a machine such as the Cambridge rocking microtome or with one of the many patterns of sliding microtome the latter give better sections but their successful employment requires more practice

The sections are floated on a deep dish of water hot enough to make them lie quite flat on its surface but not so hot as to melt the paraffin

A few of the thinnest sections are picked up on the middle of perfectly clean slides

The paraffin block is then placed in a numbered box and kept for reference

The slides are left leaning for a few minutes in an almost vertical position to allow the water to drain off them and are then put in an incubator at about 37°C After about 12 hours here the sections will be found to be fixed quite firmly to the slides so that they are not likely to come off during any of the ordinary staining processes

When difficulty is experienced in keeping sections on slides they should be mounted on albuminised slides prepared as follows —

Shake up the white of a new laid egg and filter through a moistened filter paper Add to the filtrate an equal part of glycerine, and if the mixture is to be stored for future use add a little thymol

Cover slides with a thin layer of the mixture just before they are to be used

Quicker paraffin process The routine paraffin method as described above takes from 5 days to a week but the process can be hastened by placing a portion of fresh tissue not more than 3 mm thick in a strong fixative such as formol alcohol made up as follows —

Formalin (40 per cent formaldehyde)	10 c c
Absolute alcohol	90 c c

The tissue is left there for about 12 hours and is then transferred to absolute alcohol after which it is put through as in the routine method. Sections can thus be ready on the second day after the receipt of the specimen

Paraffin sections can be obtained in one day by hastening up the process still more —

Cut a small bit of tissue about 2 mm thick and leave it for $\frac{1}{2}$ hour in formol alcohol in the paraffin oven at 60° C

Leave for 15 minutes in the paraffin oven in each of two changes of absolute alcohol which has been kept in a stock bottle over anhydrous copper sulphate

Leave in chloroform for 30 minutes in oven

Next for 10 minutes in each of three baths of wax in the paraffin oven if necessary heating the last bath until it no longer smells of chloroform

Embed cut sections and mount them on albuminised slides

Warm slides carefully over a Bunsen burner for 5 or 10 minutes

Leave slide in xylol to remove paraffin

Remove xylol with absolute alcohol

Stain with Ehrlich's hæmatoxylin in incubator for 10 minutes. Continue as in method given on p 470

Fixation in Zenker's fluid This is one of the many fixing fluids that may be used instead of formalin. Its composition

is —

Potassium bichromate	2.5 grams
Sodium sulphate	1 gram
Corrosive sublimate	5 grams
Water	100 c c

Add 5 c c glacial acetic acid just before use

Thin portions of tissue are left in the fluid for 12 to 24 hours and no longer

Wash in running water for 12 to 24 hours

Next in 70 per cent alcohol and so on to paraffin wax, as after fixation in formalin

After sections have been cut and treated with xylol and absolute alcohol, they must be treated with 1 per cent iodine or Lugol's solution for 5 minutes to remove excess of corrosive sublimate. The excess of iodine is removed by treating with 5 per cent sodium thiosulphate for 5 minutes or with 96 per cent alcohol till sections are clear. After the sodium thiosulphate the sections are washed in water for 5 or 10 minutes. The sections may then be stained.

For some stains it is advisable to have sections of tissue that has been fixed in Zenker's fluid. If however, the tissue has already been fixed only in formalin, the same results can sometimes be obtained by "Zenkerising" the sections after they have been cut. The paraffin is removed and the sections are left in Zenker's fluid for 24 hours and are afterwards treated with iodine and sodium thiosulphate in the same way as has already been described for fixation of tissue with Zenker's fluid.

Decalcification of tissue Bone or calcified tissue must be decalcified before microscopic sections can be cut.

The ordinary method which is suitable for the diagnosis of the majority of diseases, is to cut with a fretsaw or coping saw a portion of the tissue not more than 4 mm thick and, after a preliminary 24 hours or more fixation in 4 per cent saline formaldehyde, place it to be decalcified in 5 per cent nitric acid. The recognition of the termination of decalcification can be made by testing the tissue with a needle for the absence of grittiness or, more certainly, by taking X ray photographs. The necessary time in nitric acid varies from hours to days. The tissue is then left in 5 per cent sodium sulphate for twice the length of time it was in the nitric acid. It is then left in 4 per cent saline formaldehyde for 24 hours, washed in running water for 24 hours and carried forward by the paraffin method.

The routine staining of paraffin sections Each portion of tissue taken for microscopical examination should be stained by the first two methods described later, hæmatoxylin with eosin and Weigert's iron hæmatoxylin with Van Gieson's mixture.

If this practice is followed it will make histological diagnosis easier than if only one staining method is used as a routine

A set of the following staining solutions and other fluids should be kept in tightly corked glass staining pots xylol, absolute alcohol, acid alcohol (1 c c of hydrochloric acid in 100 c c of 70 per cent alcohol) Ehrlich's acid hæmatoxylin, eosin and Van Gieson's stain The slides can be immersed in the pots, so that the fluids and stains can thus be used more conveniently and repeatedly

The Weigert's iron hæmatoxylin, which is kept in two solutions, to be mixed just before use, must be poured over the sections on the slides The other stains and reagents used in the following staining methods are not likely to be in such constant use and are better kept in drop bottles

The preparation of the staining solutions is described in Chapter XI

To stain with hæmatoxylin and eosin Place slide in pot of xylol to dissolve the paraffin, about 1 minute

Place in pot of absolute alcohol to remove xylol, about 1 minute

Leave in pot of Ehrlich's acid hæmatoxylin for about 30 to 60 minutes

Leave in running tap water for about 10 minutes, till section is blue

Dip in pot of acid alcohol several times, till section turns pink

Running tap water again for 10 minutes

Leave in pot of watery solution of eosin for 5 minutes

Running tap water for about 10 minutes

Blot section with clean blotting paper

Place in absolute alcohol for 1 or 2 minutes

Place in xylol for 1 or 2 minutes

(When the slide comes out of the xylol the section should be transparent If it appears cloudy or opaque, this means that the alcohol has become diluted, and the section must go back into absolute alcohol and then into xylol again)

Mount in Canada balsam

Staining with the Ehrlich's hæmatoxylin can be done for less time, say, 10 minutes, but the results are not so good Hæmalum or Delafield's hæmatoxylin may be used, but the best results are obtained by staining with Ehrlich's acid hæmatoxylin for 1 hour

Weigert's iron hæmatoxylin and Van Gieson's mixture
Xylol Absolute alcohol

Stain with a freshly made mixture of equal parts of solutions A and B of Weigert's iron hæmatoxylin for 30 to 45 minutes

Wash in running tap water for 15 minutes

Differentiate in acid alcohol till section is of a pinkish slaty grey colour—usually about 30 seconds

Running tap water for 10 or 15 minutes

Blot section carefully

Stain with Van Gieson's mixture for 3 or 4 minutes

Rinse rapidly in tap water and blot

Dehydrate rapidly in the pot of absolute alcohol Xylol

Mount in Canada balsam which contains a few crystals of salicylic acid

Collagen fibre (fibrous tissue) is stained red

The Weigert's iron hæmatoxylin may be left on the sections for 10 minutes only but staining for 45 minutes is better

Staining elastic fibres with Weigert's elastic stain This is of the greatest help in the study of changes in blood vessels for instance when searching for arteritis in gummata

Xylol Absolute alcohol

Stain with fresh Weigert's elastic stain for 30 minutes or with Hart's elastic stain for 12 hours

Differentiate in absolute alcohol till all the section except the elastic fibres is pale grey or almost colourless If absolute alcohol fails to produce this effect dip the section a few times into acid alcohol

Rinse in distilled water

Stain with a half saturated watery solution of neutral red for 2 or 3 minutes

Rinse in distilled water

Dehydrate rapidly in absolute alcohol

Xylol Mount in Canada balsam

Elastic fibres should be stained blue black

Weigert-Gram method for organisms in sections Xylol Alcohol

Stain with aniline gentian violet for 5 minutes

Wash off excess of aniline gentian violet with Gram's iodine solution and leave the section covered with Gram's iodine for 5 minutes

Blot section with a clean filter paper

Differentiate by washing section with aniline xylol (aniline oil, 2 parts, xylol, 1 part) till it is yellowish grey

Wash aniline xylol off with xylol

Very rapid wash with absolute alcohol

Blot

Counterstain with half saturated watery solution of neutral red for 15 seconds

Blot

Dehydrate very rapidly with absolute alcohol

Xylol Canada balsam

Gram positive organisms are stained blue Stratum corneum of epidermis is stained blue and is not decolorised even by much alcohol, this stain therefore may be of assistance in the recognition of keratinised cells, as for example when there is a doubt whether a carcinoma is or is not a squamous carcinoma Mitotic figures and the granules of mast cells are also Gram positive and sometimes also degenerating nuclei

Staining sections for tubercle bacilli. Xylol Absolute alcohol

Ehrlich's acid hæmatoxylin 1 hour

Tap water 30 minutes

Carbol fuchsin 30 to 60 minutes in incubator

Acid alcohol 1 minute in incubator

Wash well in water

Leave in a solution of 1 part of a concentrated watery solution of lithium carbonate to 10 parts of water till section is blue

Wash in water for 5 to 10 minutes

Dehydrate with absolute alcohol

Xylol Mount in Canada balsam

Tubercle bacilli are stained red

Testing for iron pigment in sections Xylol Absolute alcohol

Leave 2 per cent potassium ferrocyanide on the section for 5 minutes

Pour on 1 per cent hydrochloric acid and heat the slide gently over a Bunsen burner

Mount in the hydrochloric acid

Iron-containing pigment is blue Melanin remains unaltered This stain is therefore useful in distinguishing between melanin and pigmentation from old hæmorrhage for example in a neoplasm

Muci-carmin stain for mucus Xylol Alcohol

Ehrlich's acid hæmatoxylin 30 to 60 minutes

Tap water 10 minutes

Acid alcohol till pink

Tap water 10 minutes

Stain with muci carmine solution for 15 minutes

Rinse section rapidly in water

Dehydrate in absolute alcohol

Xylol Canada balsam

Mucus is stained red This stain is useful for the identification of mucus in mucoid (so called "colloid") carcinoma

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